

ACTIVITIES OF THE WISTAR INSTITUTE APRIL, 1917, TO APRIL, 1919

A STATEMENT BY THE ADVISORY BOARD

At the fourteenth annual meeting of the Advisory Board of The Wistar Institute, held on April 20 and 21, 1919, in Philadelphia, it was decided that, owing to the relations of the Institute to other scientific institutions, societies and individuals, its activities should be given greater publicity.

The annual meeting of 1918 having been omitted, it was decided that a report on the Institute's activities should be made by the Advisory Board and should cover the two-year period from April, 1917, to April, 1919.

The general scientific policy of the Institute as outlined at the first meeting of the Advisory Board, held on April 11 and 12, 1905, was that the principal object of the Institute should be research; that the research should develop first in the field of neurology, and second in comparative anatomy and embryology. It was maintained by the Advisory Board that the museum provided for in the deed of trust should grow as a result of researches pursued in the laboratory and not as an independent activity.

Ten years later the principles formulated at that first meeting were again subjected to a careful examination by the Advisory Board in conjunction with the Board of Managers and in the light of what had been accomplished were once again approved as a basis upon which the Institute should continue activities. At the April meeting in 1917, it was demonstrated to the satisfaction of the Advisory Board that a line of anatomical research of unusual importance had been well organized and established, and was yielding valuable scientific results.

FOUNDATIONS FOR RESEARCH IN NEUROLOGY

A carefully outlined programme of intensive neurological research had produced certain very definite and important results during the preceding ten years, and had opened the way for greater and more rapid progress in this field. The growth of the brain and spinal cord of the rat from birth to maturity had been determined, also the growth of the larger divisions of the brain during the same interval. In addition, the growth of the entire body and of the principal viscera and glands (twenty in all) had been determined.

Comparison had been made of the results obtained on the rat with the corresponding results, on man, so far as the latter were available.

It had been found that the metabolic rate in the rat was thirty (30) times as rapid as in man and the span of life one-thirtieth—three years of rat life corresponding to ninety years of human life. When the rat and man of equivalent ages, thus determined, were compared they were found to be in the like phase of the span of life. Such correspondences had been found in the phases of the growth of the brain in weight; in the percentage of water in the brain (on age); in the cessation of cell division in the cerebellum; in the time of the attainment of the full thickness of the cerebral cortex; in the appearance of the cell bodies of full size in various localities.

Outside of the nervous system, the general similarity of the phases of body growth in the two species had been shown by the curves plotted; and the relatively more rapid growth of the female in the prepubertal period and the occurrence of the menopause at equivalent ages had been observed. Attention had been called to the fact that the determination of these relations furnished for the first time a rational basis for the comparison of the results obtained on an animal with those obtained on man, and that there is the further advantageous circumstance that the range of diet in the rat is almost as great as for man.

These facts taken together justified the further use of the rat in this work and facilitated the study of a number of questions—especially those involving experimental modifications—which could not be applied directly to the human subject.

By other researches the composition of the nervous system in terms of water and solids at different ages had been established and the chemical differentiation of the system with age had been followed—in the latter instance for the brain only. The fact that age is the condition most important for the percentage of water had been established. It had also been shown that the active portion of the nerve tissue does not lose water with advancing age, but that the loss of water in the brain and cord is brought about by the progressive formation of myelin—which has a low water content. This demonstration was new.

The growth of the neuron, the growth of the cell bodies, of the Purkinje cells in the cerebellum and of several types of cells in the cerebral cortex had been studied.

The nerve fibers in the spinal nerves had been shown to increase in diameter long after the cells of origin have reached their full size, and in the case of the myelinated fibers, the relations of the sheath to the axis had been carefully determined (in one nerve). The steps in the regeneration of an interrupted nerve with the remarkable overgrowth at the level of repair had been recorded with great precision.

The studies of the peroneal nerve and of the olfactory bulbs served to establish the fixity (within biological limits) of the number of neurons constituting the nervous system of the rat, which means that the nervous system in this species is formed by a definite number of cell divisions, i.e., a definite number of cells. The same is probably true for man, but there are no data.

The fact had been established that at birth cell division is nearly complete in most parts of the nervous system (rat), but in the cerebellum it continues for two weeks or more after birth. In man it continues for the equivalent length of time also. In experiments undertaken to modify the growth of the nervous system it had been shown that exercise improves growth—while the various forms of underfeeding, thus far tried, merely retard growth temporarily.

A study of the Norway and of the albino rats in Vienna, Paris and London, as compared with the two forms in Philadelphia,

had shown that the Norways from all stations are similar to one another, and the Albinos are also similar to one another.

Between the two forms, however, several constant differences had been shown. The most important in this connection is the observation that the relative brain weight in the Norway rat is 12 per cent to 15 per cent greater than in the Albino. This difference is attributed to the fact that the Albino lives under the conditions of domestication. The structural details of the nervous system in these two forms have been compared in several instances, but no generalizations thus far reached except that the larger brain of the Norway appears to be due to the larger size of the neurons—the number remaining the same as that of the Albino.

These investigations have yielded a considerable body of fact touching the growth of the nervous system in the rat—and wherever they could be tested, the results apply to man also.

With these foundations the continuation of the neurological research, during the early part of the two-year period of which we are speaking, was one of rapid progress. War conditions interfered in several ways, owing to the difficulties of obtaining equipment, in maintaining the rat colony and in securing assistance of various kinds.

As might be expected, a complete and successful plan of work has produced results which have attracted the attention of others interested in the growth phase of neurology. The original Staff of six members has been increased to ten, and during 1917 ten guests and in 1918 twelve guests were engaged in research in the Institute laboratories.

With a total of twenty-two persons engaged in research, the facilities of the Institute were taxed to their limit and it was impossible to accept others who applied.

The war conditions had much to do with the influx of guests in the laboratories.

Owing to the demands presented by guests in the laboratories the field of research has been extended at times somewhat beyond the limits of neurology, yet such studies have usually been planned so as to add to our knowledge of the albino rat.

The bibliography which follows will give in detail the finished results of the two years' work.

NEUROLOGICAL RESEARCH

The study of the proportion of myelin in the peripheral nerves and in the corpus callosum by chemical methods has been completed and shows the important result that myelination is quantitatively similar in both the central and the peripheral system.

A study of the human cortex from birth on, with special reference to the stratification of the cortical layers and the size and number of cells at different fixed localities, was planned and begun. The object of this work is to obtain a series of norms for the cortex of man and to use these norms for the interpretation of the conditions found in defectives. The first study completed in this series was a quantitative study of the Purkinje cells in normal, subnormal and senescent human cerebella, with some notes on the functional localization. The Purkinje cells were found to be fewer in the subnormal and senescent brains than in the normal, and after the fourth decade the number of these cells diminishes in the normal person as a function of age and independently of the mental grade of the individual.

A study on transplantation of the cerebral cortex begun in another laboratory was published showing survival of the transplanted cells, but no functional connections were established.

The paraflocculus was studied and found relatively much smaller in the albino rat than in the wild Norway, the details of this difference in terms of the thickness of cerebellar cortex and number and size of Purkinje cells remain to be determined. The paraflocculus was found to behave like that other semi-isolated brain mass, the olfactory bulb, under modifying conditions.

Observations on the number, size and axis-sheath relations in the largest fibers of the peroneal nerves in the inbred albino rat and on the modifications of the fibers due to pneumonia and to electrical stimulation were completed.

Attention has been directed to the determination of whether or not the effects of section of the peroneal nerve are extended

to all the other peripheral nerves or only to the corresponding nerve of the opposite side. This work remains to be completed.

For the purpose of following the formation of the myelin sheaths, Sudan III was fed to a series of rats. The substance was not deposited in the nervous system, hence could not be used as an indicator for myelin formation. It did, however, arrest growth, or cause an atrophy in the growth of the thymus gland. As concerns the thymus gland, this part of the research has been turned over to another laboratory where the thymus is being studied.

The most important neurological study completed during the two-year period was Dr. Sugita's comparative studies on the growth of the cerebral cortex. This was begun in 1915 and completed in 1917, and is presented in eight papers.

The data show that the number of neurons is the same in the brain of the Norway and the Albino, i.e., cell number is a species character; and that the number and size of cells and cortical thickness characteristic of the mature brain had all been attained at twenty days of age—the weaning time for the rat. The equivalent age for man is fifteen months, at which time we should expect the human cortex to be similarly complete.

The work of Dr. Sugita has been carefully followed and revised by Dr. Donaldson who has prepared a brief summary of the whole series of papers which has been published as introduction to this investigation.

Studies on the growth of the cell bodies in the third cervical sympathetic ganglion of the rat are under way. This is the first on the growth of the sympathetic system made at the Institute.

A study of the growth of the fibers in the corpus callosum from birth to maturity, a research beset with technical difficulties, is being successfully accomplished.

Attention has been directed to the growth of the cell body in relation to the growth of its axon or fiber. In a study of the fifth segment of the spinal cord with its nerve roots and ganglia from albino rats of various known ages, it was shown that the cell bodies reach their maximum size early, while the fibers con-

tinue to grow for nearly the first year of life. Interesting relations between the volume of the cell bodies, the sectional area of the fiber and the area of body surface have been found.

OTHER STUDIES IN THE NEUROLOGICAL LABORATORY

During the two-year period studies have been made on the hypophysis, after castration, with positive results, and the description of a new structure in the cytoplasm in one class of these cells.

The testis has been the subject of a careful histological study made under a new and superior technique which preserves the normal relations of the parts of the testis. This technique has been used already in the study of the dividing cells of the cerebellar cortex.

Careful studies are being made on the number of ova in the ovary of the rat at different ages and on the number found in the remaining (hypertrophied) ovary after one ovary has been removed.

A study of the influence of isolated ovaries on body growth showed that ovaries isolated within the body of the animal still continue to exert their growth control.

An investigation of the permeability of the placenta has been carried on during the last half year, but no report can yet be made.

An elaborate study on the growth of the skeleton involving a large mass of data and computations is nearing completion. This will complete the growth studies on the various systems outside of the nervous system.

A study made of the cell elements of the rat's blood raises questions which indicate the need of a more complete description of the cellular composition of the normal blood at different ages.

SEROLOGICAL STUDIES

Determination of the refractive index of the rat's blood at different ages was made, and these studies reveal a very marked and regular change in the index with age, accompanied by

characteristic modifications related to the first suckling, weaning and to puberty. The value of the refractive index in adult man and the adult rat is the same. This study gives one more similarity between the rat and man, and another illustration of the importance of age in relation to functional processes. By determination of the refractive index of serum, following the fractional precipitation of its contained protein, a curve has been established for normal human blood. It is found that syphilitic blood, blood from tubercular subjects, from cases of Bright's disease, and from cases of cancer present characteristic curves when determined by this method. There is here promise of some very important clinical applications, as the technique is extremely simple.

The chemical study of blood serum has yielded some very important and interesting results. The protein fraction containing the complement has been isolated and proven to contain all the complement. By the methods devised the complement may now be separated from serum and used to reactivate inactivated serum. At the close of our two-year period, April 20, 1919, other serum elements were receiving attention.

A study has been made on the relation between agglutination and refractive index in the serum of the albino rat. Tests show that the serum of older rats, or serum of higher refractive index, agglutinates the corpuscles of the younger rats. The reverse relation did not hold, but it was found that the younger (more dilute) serum causes a swelling of the older corpuscles to an enormous degree; an escape of hemoglobin was in several instances noted.

In a study of anti-pig hemolysin, it was found to be present in the majority of cases of pregnancy and during puerperal stages, while in non-breeding females it was found in fewer cases and still less frequently in normal males.

In order to complete this investigation, the following experiments are now under way:

a. To determine whether the natural anti-pig hemolysin is present in all rats;

b. To determine whether the hemolysin suddenly appears during pregnancy, or the puerperal stage, and then disappears.

The experiments so far made indicate that these natural hemolysins may appear during pregnancy when they have been absent previously.

A number of other serological investigations have been completed as will be shown in the bibliography.

By a new technique, devised by Dr. S. Hatai, the non-protein nitrogen in various proteins of the central nervous system may be easily and accurately determined. A study of the distribution of the non-protein nitrogen in the central nervous system of the albino rat was made, showing that non-protein nitrogen is present in proportion to the active cell substance and independent of the amount of myelin, and, further, that it is alike for both the cell bodies and the axons.

A study of the non-protein nitrogen content in the brain of albino rats during the twenty-four hours after feeding shows that the non-protein nitrogen varies regularly during the twenty-four hours following a meal, and the data when plotted give a periodic curve.

A study of the non-protein nitrogen in the brain of a fish (the gray snapper) showed the partition to be essentially similar to that in the brain of the rat.

While the research in neurology has been most comprehensively planned and most intensively followed by the majority of investigators at the Institute, at the same time this report would be incomplete without mention of the other lines of important research which have engaged the attention of some members of the Staff.

GENETICS

Dr. Helen Dean King has continued her inbreeding experiments which have now reached the thirtieth generation of animals, obtained by the mating of brother and sister, the closest possible inbreeding. The objects of this experiment are to determine the effects of inbreeding on growth and variability in body weight, on the fertility, constitutional vigor and sex ratio. As very

definite results were obtained when the twenty-fifth generation was reached, it was thought advisable to publish the records up to this point, which comprise over twenty-five thousand rats. This has been done.

Dr. King's results show that the closest form of inbreeding in the albino rat—the mating of brother and sister from the same litter—is not necessarily harmful to body growth, to fertility, or to constitutional vigor, provided the best animals are selected for breeding from a relatively large number. Selection seems to counteract the tendency which inbreeding may have to intensify defective traits.

These investigations show that adverse conditions of environment and food have greater detrimental effects than selective inbreeding, and that such adverse conditions do not alter the genetic constitution of the animal, since it resumes its normal growth and fertility when favorable environmental and nutritive conditions are restored.

It appears from these investigations that the sex ratio in the rat is amenable to selection, since the inbred strain has been separated into two distinct lines, one showing a high sex ratio and the other showing a low sex ratio. This selection, however, is limited, as no cumulative effects were obtained.

As regards the variability of body weights, shown in the early generations of inbreeding, it appears to be impossible to draw any definite conclusions until environmental and nutritive conditions can be controlled or made uniform.

While the inbreeding experiment is being continued, at this stage, the plan of conducting it was modified in order to obtain information regarding the value of crossing inbred lines and of reversing selection in attempting to influence the sex ratio.

During the period of our report Dr. King has been conducting experiments to determine:

The effect of age of parents on the vitality of their young.

The physiological relation between coat color and body growth in extracted strains of rats.

The effects of hybridization on the sex ratio of the rat.

Inheritance of structural anomalies in the rat.

A number of minor problems are also receiving attention.

EMBRYOLOGY

The research in embryology during the two year period has been of unusual interest. Prof. Carl Hartman, of the University of Texas, assisted by Dr. C. H. Heuser, of The Wistar Institute, having collected in Texas a very complete series of opossum eggs and embryos, began a series of studies on this unique type. One hundred and seventy-nine eggs and embryos were sectioned and mounted.

The first paper by Dr. Hartman, dealing with the early stages, has been published. Six hundred and forty-one normal opossum eggs, in a closely graded series, formed the basis of this study from ovarian and tubal ova through maturation, cleavage, blastocyst formation, entoderm formation, and the bilaminar stage to the first proliferation of mesoderm.

This investigation shows that entoderm mother cells differentiate in one half of the blastocyst wall at about the sixty-celled stage, round up and pass into the blastocyst cavity where they give rise to the entoderm.

The bilaminar stage is fully treated and its growth and development to the first anlage of the primitive streak are described.

In this paper Dr. Hartman has discussed the elimination of the yolk; the origin of the crossed arrangement of blastomeres in the four-celled stage; typically eutherian and marsupial characters in the early ontogeny of the opossum; the origin of the embryonic and the trophoblastic areas each from one of the first two blastomeres. He observes that the reduced number of chromosomes is twelve.

Dr. C. H. Heuser is studying the later stages of opossum development and has completed much of the technical details in preparing sections and models for these studies.

Wax plate reconstructions have been made of the brain, digestive and respiratory tracts, jugular lymph sac, and the heart. That of the alimentary tract includes the thyroid gland, the thymus, the hypophysis, the nasal cavities, the pancreas, the gall bladder, the ureter and pelvis of the kidney, the stalk of the allantois, and enough of the Wolffian duct to show its connection with the cloaca.

Minute dissections of embryos, done under the binocular microscope, have been made to show structure relationships.

A large portion of the technical detail has been completed to make this unique series available for further studies.

The original preparations used in the first publication are now available for further study, or for comparison at the Institute.

Embryological material of the kind here mentioned, with the drawings, photographs and models used in investigations, constitute the ideal type of museum material. It presents a vast storehouse of facts for future investigations.

BIBLIOGRAPHY

The following is a list of the 31 papers published during the April, 1917, to April, 1919, period, covering all fields of the Institute's research.

- ADDISON, W. H. F. 1917 The cell-changes in the hypophysis of the albino rat, after castration. *Jour. Comp. Neur.*, Vol. 28, pp. 441-463.
- ALLEN, EZRA 1918 Studies on cell division in the albino rat (*Mus norvegicus albinus*). III. Spermatogenesis: The origin of the first spermatocytes and the organization of the chromosomes, including the accessory. *Jour. Morph.*, Vol. 31, pp. 133-185.
- CONROW, SARA B. 1917 A six-legged rat. *Anat. Rec.*, Vol. 12, pp. 365-370.
- DONALDSON, H. H. 1918 Introduction to the "Comparative studies on the growth of the cerebral cortex, by Dr. Naoki Sugita." Parts I and II in *Jour. Comp. Neur.*, Vol. 28; parts III to VIII in Vol. 29.
- DONALDSON, H. H. 1918 A comparison of growth changes in the nervous system of the rat with corresponding changes in the nervous system of man. *Proc. of the Nat. Acad. of Sciences*, Vol. 4, pp. 280-283.
- DONALDSON, H. H. AND NAGASAKA, G. 1918 On the increase in the diameters of nerve cell bodies and of the fibers arising from them—during the later phases of growth (albino rat). *Jour. Comp. Neur.*, Vol. 29, pp. 529-552.
- DUNN, ELIZABETH H. 1917 Primary and secondary findings in a series of attempts to transplant cerebral cortex in the albino rat. *Jour. Comp. Neur.*, Vol. 27, pp. 565-582.
- ELLIS, ROBERT S. 1919 A preliminary quantitative study of Purkinje cells in normal, subnormal, and senescent human cerebella, with some notes on functional localization. *Jour. Comp. Neur.*, Vol. 30, pp. 229-252.
- GREENMAN, M. J. 1917 The number, size and axis-sheath relation of the large myelinated fibers in the peroneal nerve of the inbred albino rat—under normal conditions, in disease and after stimulation. *Jour. Comp. Neur.*, Vol. 27, No. 3, pp. 403-420.

- HATAI, S. 1917 On the condition of the albino rats on East Key and Garden Key in the Dry Tortugas. Year Book No. 16 of the Carnegie Institution of Washington, pp. 179-182.
- HATAI, S. 1917 Metabolic activity of the nervous system. Jour. Comp. Neur., Vol. 28, pp. 361-378.
- HATAI, S. 1918 The brain weight in relation to the body length and also the partition of non-protein nitrogen, in the brain of the gray snapper (*Neomaenis griseus*). Proc. of the Nat. Acad. of Sciences, Vol. 4, pp. 19-21.
- HATAI, S. 1918 Metabolic activity of the nervous system. II. The partition of non-protein nitrogen in the brain of the gray snapper (*Neomaenis griseus*) and also the brain weight in relation to the body length of this fish. Jour. Comp. Neur., Vol. 29, pp. 41-59.
- HATAI, S. 1918 On several effects of feeding small quantities of Sudan III to young albino rats. Jour. Exp. Zool., Vol. 26, pp. 101-117.
- HATAI, S. 1918 On the weight of the epididymis, pancreas, stomach and of the submaxillary glands of the albino rat (*Mus norvegicus albinus*) according to body weight. Am. Jour. Anat., Vol. 24, pp. 71-89.
- HATAI, S. 1918 The refractive index of the blood serum of the albino rat at different ages. Jour. of Biol. Chemistry, Vol. 35, pp. 527-552.
- KING, HELEN D. 1918 Studies on inbreeding. I. The effects of inbreeding on the growth and variability in the body weight of the albino rat. Jour. Exp. Zool., Vol. 26, pp. 1-54.
- KING, HELEN D. 1918 Studies on inbreeding. II. The effects of inbreeding on the fertility and on the constitutional vigor of the albino rat. Jour. Exp. Zool., Vol. 26, pp. 55-98.
- KING, HELEN D. 1918 Studies on inbreeding. III. The effects of inbreeding, with selection, on the sex ratio of the albino rat. Jour. Exp. Zool., Vol. 27, pp. 1-35.
- KOCH, W. AND KOCH, M. L. 1917 Contributions to the chemical differentiation of the central nervous system. IV. The relative amount of sheathing substance in the corpus callosum and intradural nerve roots (man and dog). Jour. of Biol. Chemistry, Vol. 31, pp. 395-410.
- SUGITA, NAOKI 1917 Comparative studies on the growth of the cerebral cortex. I. On the changes in the size and shape of the cerebrum during the postnatal growth of the brain. Albino rat. Jour. Comp. Neur., Vol. 28, pp. 495-510.
- SUGITA, NAOKI 1917 Comparative studies on the growth of the cerebral cortex. II. On the increase in the thickness of the cerebral cortex during the post-natal growth of the brain. Albino rat. Jour. Comp. Neur., Vol. 28, pp. 511-591.
- SUGITA, NAOKI 1918 Comparative studies on the growth of the cerebral cortex. III. On the size and shape of the cerebrum in the Norway rat (*Mus norvegicus*) and a comparison of these with the corresponding characters in the albino rat. Jour. Comp. Neur., Vol. 29, pp. 1-9.
- SUGITA, NAOKI 1918 Comparative studies on the growth of the cerebral cortex. IV. On the thickness of the cerebral cortex of the Norway rat (*Mus norvegicus*) and a comparison of the same with the cortical thickness in the albino rat. Jour. Comp. Neur., Vol. 29, pp. 11-39.

- SUGITA, NAOKI 1918 Comparative studies on the growth of the cerebral cortex. V. Part I. On the area of the cortex and on the number of cells in a unit volume, measured on the frontal and sagittal sections of the albino rat brain, together with the changes in these characters according to the growth of the brain. V. Part II. On the area of the cortex and on the number of cells in a unit volume; measured on the frontal and sagittal sections of the brain of the Norway rat (*Mus norvegicus*), compared with the corresponding data for the albino rat. Jour. Comp. Neur., Vol. 29, pp. 61-117.
- SUGITA, NAOKI 1918 Comparative studies on the growth of the cerebral cortex. VI. Part I. On the increase in size and on the developmental changes of some nerve cells in the cerebral cortex of the albino rat during the growth of the brain. VI. Part II. On the increase in size of some nerve cells in the cerebral cortex of the Norway rat (*Mus norvegicus*), compared with the corresponding changes in the albino rat. Jour. Comp. Neur., Vol. 29, pp. 119-161.
- SUGITA, NAOKI 1918 Comparative studies on the growth of the cerebral cortex. VII. On the influence of starvation at an early age upon the development of the cerebral cortex. Albino rat. Jour. Comp. Neur., Vol. 29, pp. 177-240.
- SUGITA, NAOKI 1918 Comparative studies on the growth of the cerebral cortex. VIII. General review of data for the thickness of the cerebral cortex and the size of the cortical cells in several mammals, together with some postnatal growth changes in these structures. Jour. Comp. Neur., Vol. 29, pp. 241-278.
- WHITING, P. W. AND KING, HELEN D. 1918 Ruby-eyed dilute gray, a third allelomorph in the albino series of the rat. Jour. Exp. Zool., Vol. 26, No. 1, pp. 55-64.

EXTENSION OF THE INSTITUTE'S RESEARCH

For some years past the Institute has been cooperating with the Training School at Vineland, N. J., in the study of certain phases of neurology presented by the mentally defective.

Early in 1918 this cooperative arrangement was more carefully defined and arrangements were made for increasing the Institute's staff in order to take up the problems presented.

In making a study of abnormal development the methods of procedure involve the determination and measurement of so many normal structures and processes that the final results will have a much wider application to the welfare of normal individuals than to the mere solution of the problem of the feeble-minded.

To do this work it was planned to maintain at the Vineland Training School a staff fitted to follow and record the growth,

behavior (psychological) and clinical history of the four hundred and ninety children in the institution. This is being done.

At the same time it was planned to extend the present work on the growth of the nervous system of the rat, as the results obtained with the rat are applicable to man, and also to lay special stress on the collection of data on the normal human nervous system. This work is also well under way.

When these data are obtained, the deviations of the defectives from the normals can be directly determined, and some inferences made concerning the causes of the defective state and the possible part played by growth conditions in bringing about results. This comparison will undoubtedly suggest studies on other organs and tissues and further experimental studies which can be followed out with the rat in the laboratory. It cannot be too strongly stated that no experimental work worthy of the name can be done on man, that experimental tests are absolutely necessary for real progress, and that the rat is a form which not only can be used, but which gives results that can be applied to man as indicated elsewhere in this report.

Emphasis should be put also on the fact that the normal data—animal or human—which are obtained have the widest application, while the defectives form one class only of the pathological cases, all of which must be referred to the standard data.

In this cooperative arrangement the investigations will group themselves into three main divisions:

1. The clinical or all those observations concerned with the living individual to be conducted at the Training School.

2. The postmortem or laboratory observations—studies and analyses of organs and tissues after death, to be commenced at the Training School and completed at The Wistar Institute.

3. Experimental studies with living animals to determine normal processes and deviations under abnormal conditions. These studies will be conducted at The Wistar Institute. Here also will be made the records on the normal human nervous system.

Special attention is being directed to the study of the nervous system, considering for the present the brain and the causes

which have modified its development. Studies of the brain will embrace:

a. Observations on the proportions of the different divisions of the brain—cerebrum, cerebellum, stem.

b. Cytological study of cortex of cerebrum.

c. Cytological study of cortex of cerebellum.

d. Chemical examinations of these divisions of the brain.

The third group of observations will comprise studies on a living mammal (rat) to determine by experimental methods normal growth processes and the effects on normal processes of various modifying influences, such as removal of the various ductless glands, under-feeding and poisoning with various reagents, with the object of producing experimentally, if possible, some of the conditions found in defective children.

Three new members and two technicians were added to the staff to carry on the Institute's part of the work planned.

The results accomplished are reported in more or less detail elsewhere in this report under the heading of Research in Neurology.

It is proper to mention here that the arrangements for extending the neurological research in cooperation with the Vineland Training School have been made possible through the wisdom and generosity of Mr. Samuel S. Fels, of Philadelphia.

The laboratory equipment furnished in part by the Vineland Training School has been very much increased and funds for the support of investigators supplied by Mr. Fels.

THE RAT COLONY

The use of the albino rat for accurate quantitative anatomical analysis does not appear to present any unusual difficulties. The production of a standard rat, however, is somewhat more difficult than at first appears.

This animal is extremely responsive to its food and treatment. For some years past it has been the practice at The Wistar Institute to raise rats on a food consisting chiefly of table scraps from nearby restaurants. During the food-conservation

campaign of the past two years, table scraps declined in rat-food value to a low level and it became necessary to purchase grains and other foods for the colony. After some unfortunate experiences with toxic foods and insufficiently balanced rations, the food question was solved. For the most accurate, scientific information on foods for the albino rat the Institute is indebted to Prof. E. V. McCollum, of Johns Hopkins University.

During the two-year period of which we are speaking the colony furnished nearly thirty thousand animals to Government and other outside laboratories, or a product of one animal every thirty-five minutes of the entire period.

Those who have had occasion to observe the use of a so-called standardized animal for accurate biological research are impressed with the importance of greater care in the breeding and rearing of animals for research purposes.

The Wistar Institute is now devoting time, energy and thought to this subject with the object of producing a perfectly healthy animal which at any given age is a known quantity in every essential detail.

The results of greater care and attention in the production of a standardized animal are being realized in almost every phase of the Institute's research where accuracy is essential.

THE MUSEUM

The most important additions that have been made to the museum during the period is the collection of opossum eggs and embryos, one hundred and seventy-four of which are already mounted on glass for study, the drawings and models resulting from studies of these embryos and a series of mounted rat skins showing mendelian inheritance of coat color.

The neurological laboratory is also the source of a continuous addition to the histological collections.

JOURNALS

The journals published by the Institute are as follows:

Journal of Morphology issued 4 times a year

The Journal of Comparative Neurology

. issued 6 times a year

The American Journal of Anatomy

. issued 6 times a year

The Anatomical Record issued monthly

The Journal of Experimental Zoölogy

. issued 8 times a year

The American Anatomical Memoirs

. issued at irregular intervals

The two main objects in bringing these periodicals together under one publishing management are:

1. To publish and distribute research effectively and economically.

2. To furnish conditions for the control of such publications by those interested in the promotion of science.

Soon after the journals were brought together an organization was perfected for the proper handling of manuscripts and proofs, for extending the distribution, for the keeping of records and for attending to many details connected with the publication of 150 to 200 manuscripts each year.

All extra copies of journals held for future distribution—about 100 copies of each issue—are stored in the Institute's fire-proof building. All subscription lists are kept and all mailing is done at the Institute, an arrangement which has given the Institute an opportunity of studying the requirements of libraries and laboratories, of making exchanges and subscription combinations, and of aiding in the dissemination of scientific literature in ways which otherwise would have been difficult, if not impossible.

With the men and institutions of the world, to whom these various publications are distributed, The Wistar Institute maintains more or less active contacts. This has been found essential for the introduction of new literature, for making exchanges and in a variety of ways.

Attention was given to improvements in paper and methods of bookmaking—improvements which are carried as far as funds would permit. The problem of better illustrations still remains to be solved.

A fixed date of publication was established and rigidly followed, thus avoiding the weeks and even months of delay as in former times.

The American Association of Anatomists at first contributed \$4.50 per year per member in support of *The American Journal of Anatomy* and *The Anatomical Record*, and received these journals in return for this contribution. In 1915, this contribution was increased to \$6.50 per member without promise of additional journals.

The Institute's guarantee of permanence for the journals has shown results by the increase of subscription receipts. With all this support, however, the income was far from that required to meet the increasing demands upon the journals' space. To meet these demands, a policy of extension was inaugurated by The Wistar Institute in 1915.

It was planned to place the journals in every public and private library where they might be of service regardless of the ability of such library to pay the subscription prices. It was expected that by this method they might be brought to the attention of more persons interested and would eventually gain for themselves legitimate support as well as aid in the advancement of science.

Notwithstanding the difficulties imposed by the European War, extra funds were secured which made it possible to publish promptly all accepted papers and also to give to each member of the American Association of Anatomists not only all issues of the *American Journal of Anatomy* and *The Anatomical Record*, but also all issues of *The Journal of Comparative Neurology* and the *Journal of Morphology*.

In 1916, the American Society of Zoologists was persuaded to join in the support of these journals in return for similar journal privileges.

In 1917, each member of the American Association of Anatomists received:

- 2 volumes Journal of Morphology,
- $1\frac{3}{4}$ volumes The Journal of Comparative Neurology,
- 2 volumes The American Journal of Anatomy,
- $2\frac{1}{12}$ volumes The Anatomical Record,

or journals equivalent to a subscription value of \$56.50 in return for \$6.50 in dues paid to The Wistar Institute.

It will be seen that the actual monetary gain for the journals, arising from society contributions is very trifling.

A much more important thing, however, has been achieved—the distribution of the publications to a greater number of active investigators.

What has been accomplished in the support of these publications is indicated in the following table:

	<i>Journal receipts</i>	<i>Pages published</i>
1913.....	\$13,552.80	4060
1914.....	16,288.92	4180
1915.....	15,778.52	4660
1916.....	24,095.95	4960
1917.....	27,500.31	6768
1918.....	29,423.33	5114

These items do not include deficits paid by The Wistar Institute which averaged about \$6000 per year.

The records of costs, as compared with those of the earlier management, show that the economies of a central publication office for five journals, together with the standardization of methods of manufacture, result each year in a sufficient saving to pay the entire overhead charges of the central office. Each publication added to the combination reduces the overhead charge per journal.

The experience of The Wistar Institute thus far seems to indicate that a number of journals may be made self supporting as a group, whereas, operated independently, few if any would be entirely self supporting.

One of the most important functions of the central publication office is to relieve editors, especially managing editors, of a multitude of details incident to the printing of journals. Time is thus saved for the prosecution of research.

BIBLIOGRAPHIC SERVICE

In January, 1917, the printing of Bibliographic cards was begun and in June of the same year authors' abstracts were added to the cards.

The Bibliographic Service has been favorably received from the beginning. Constructive criticism has aided in perfecting the service. The next and most important improvement pertains to the arrangement of abstract material with the object of classifying information under well-selected subject headings in order that the Bibliographic cards may be incorporated in subject catalogues.

The Institute is now issuing abstracts for all articles appearing in the *Biological Bulletin*, published by the Marine Biological Laboratory, and will soon extend the Bibliographic Service to *The American Journal of Anthropology*, to *Endocrinology* and to *The Journal of Animal Behavior*.

During the past two and a half years special attention has been paid to the distribution of American journals in the Orient, where formerly the Institute had but two or three subscribers, and to South America, where it had but one. Numerous free copies have been sent to South America and to the Orient, and a careful analysis of the records on May 22, 1919, shows that in about two years' time ten per cent of all free copies have become full-paid subscriptions; and this is not to mention the more important achievement of placing the publications where they are doing good service in the promotion of science.

The national crisis had impressed upon the Institute's management the desirability of more intimate relations with the Republics of Latin America and with the countries of the Orient. It seemed quite possible that some good might be accomplished, not only for science, but also in promoting such international

relations, by establishing active coöperation between the scientific men of these countries and those of the United States.

Acknowledgments are due Honorable Wm. G. McAdoo, former Secretary of the Treasury and Secretary of the International High Commission; Dr. Leo S. Rowe, Assistant Secretary of the Treasury, and Mr. John Barrett, Director-General of the Pan-American Union, for valuable assistance rendered in establishing relations with the scientific institutions in Latin American countries.

The total number of subscriptions to these publications on December 31, 1909, was 1713; distributed as follows:

Journal of Morphology	188
The Journal of Comparative Neurology	231
The American Journal of Anatomy	528
The Anatomical Record	421
The Journal of Experimental Zoölogy	345

Of this 1713, 129 were free copies, 538 were distributed to members of the American Association of Anatomists at reduced rates, and 1046 were full-paid subscriptions.

Ten years later, May 22, 1919, the total number of copies of these publications distributed was 4750, apportioned as follows:

Journal of Morphology	1067
The Journal of Comparative Neurology	954
The American Journal of Anatomy	963
The Anatomical Record	959
The Journal of Experimental Zoölogy	807

An analysis of the above table, showing the details of distribution according to journal follows:

JOURNAL	NUMBER OF FULL PAID SUBSCRIPTIONS	SUBSCRIPTIONS AT ASSOCIATION OR CLUBBING RATES	COPIES SENT FREE TO INDIVIDUALS OR INSTITUTIONS
Morphology	196	595	276
Neurology	151	524	279
Anatomy	196	485	282
Record	149	527	283
Zoölogy	248	282	277

As before stated, of the original number of free copies, sent chiefly to South American and Oriental libraries, ten per cent have now become full paid subscriptions, and this percentage is gradually increasing.

The editors have been in an irregular manner chosen from laboratories and societies not connected with The Wistar Institute, and it is one of the most important points in the development of American publications that each should be national in character and not dominated by a local group.

At the present time there is a movement on foot to have the editors of The American Journal of Anatomy and The Anatomical Record elected to serve for three years by a joint committee composed of Trustees of The Anatomical Journal Trust and a Committee of the American Association of Anatomists.

It is the opinion of the Advisory Board that each journal issued by The Wistar Institute should have editors selected periodically by some national body like the American Association of Anatomists or the American Society of Zoologists. The scientific policy of each journal would then be kept in the control of representative biologists.

The Wistar Institute has followed this policy in practice as far as it has been possible. Unfortunately, there has never been any concerted action to bring into actual practice this method of controlling the scientific policy of biological periodicals.

The Wistar Institute was organized under a charter from the State of Pennsylvania to stimulate interest and pursue research in any field of biology and especially in anatomy.

It was not expected that it would go extensively into the publication of periodicals. Its total endowment income of about \$45,000 will not permit of any large expenditure on publications with all its other work to maintain.

What the Institute has accomplished in the organization of a group of zoological periodicals under a central publication office is considered by its Managers to be one of the best contributions it could make to biology, and, owing to world conditions, which have imposed upon our country an additional obligation, the Institute management feels it a duty and an opportunity to

undertake as much as its resources will permit. It has demonstrated the feasibility and desirability of a central publication office and democratic control of scientific policy.

When other and better means are provided for caring for the publication of American biological periodicals, The Wistar Institute will gladly welcome relief from this work in order that it may devote its entire energies to research.

LLEWELLYS F. BARKER

EDWIN G. CONKLIN

HENRY H. DONALDSON

SIMON H. GAGE

MILTON J. GREENMAN

GEORGE S. HUNTINGTON

G. CARL HUBER

J. PLAYFAIR McMURRICH

CLARENCE E. McCLUNG

GEORGE A. PIERSOL

Members of the Advisory Board.

Resumen por el autor, George S. Huntington.
Universidad de Columbia, Nueva York.

La morfología de la arteria pulmonar de los mamíferos.

1. Morfogénesis. El presente trabajo se ocupa de los estados ontogénicos tempranos de la arteria pulmonar en embriones de gato (de 4 a 6 mm.). Las arterias pulmonares de los mamíferos no se originan primariamente como brotes o proliferaciones de los segmentos ventrales del sexto arco aórtico y no se extienden caudalmente por un proceso de crecimiento continuo o "gemación." Se desarrollan por el establecimiento de anastomosis longitudinales entre un cierto número de derivados postbranquiales de las aortas dorsales, que se abren en el esbozo pulmonar, formando un plexo temprano pulmonar postbranquial. Este plexo es al principio completamente independiente del esbozo ventral del sexto arco aórtico, con el cual se reúne secundariamente dando lugar, en estados ulteriores, a los brotes que constituyen este vaso y que aparentemente provienen de este arco, como se describe ordinariamente. El esbozo dorsal del sexto arco (conducto de Botal) está constituido por el elemento más cranial del plexo pulmonar postbranquial. 2. Consideraciones filogénicas. Por su significación filogénica los estados ontogénicos tempranos de la arteria pulmonar de los mamíferos colocan a esta arteria en línea serial directa con los canales vasculares respiratorios de los vertebrados inferiores y completan el plan fundamental común a todos los vertebrados para la interpretación de esta porción del sistema vascular hemal. 3. Relación de los estados embrionarios descritos con la aparición de variaciones y mutaciones de la arteria pulmonar en el hombre y en los vertebrados inferiores.

Translation by José F. Nonidez
Carnegie Institution of Washington

THE MORPHOLOGY OF THE PULMONARY ARTERY IN THE MAMMALIA

GEO. S. HUNTINGTON

Columbia University

TWO TEXT FIGURES AND FIVE COLORED PLATES

In the course of an extensive investigation into the phylogeny and morphology of the vertebrate respiratory apparatus, the vascular supply of the mammalian lung required a detailed review of the early ontogenetic stages in relation to their influence on the architectonics of the developing organ. During the progress of this study certain early phases in the development of the pulmonary arteries in the cat and albino rat showed conditions which warrant an important modification of the accepted genetic interpretation of the main pulmonary arteries of the mammalia as developing through caudally directed buds or outgrowths from the ventral portions of the sixth aortic arches. Since these results bear directly on the phyletic significance of these vessels and of the mammalian respiratory tract as a whole, a preliminary excerpt from the more extensive work now nearing publication is here presented. In embryos of the cat measuring from 7 mm. up the pulmonary artery is found arising from the ventral portion of the sixth arch of each side, and descending as a complete and continuous vessel to supply the developing lung. The descriptions of these later stages fail, however, to take cognizance of a distinct antecedent phase in the development of the pulmonary artery, in which the entire vessel, prior to its subsequently attained connection with the sixth arch, is the product of a longitudinal anastomosis between a series of transversely directed ventromedial branches of the dorsal aortae caudal to their point of connection with the sixth arch. The critical stages are encountered in cat embryos of between 3 mm. and 6 mm. crown-rump measure. Considerable variation is found in individual

examples in regard to the relation between the events of this early period and the total length of the embryo, probably dependent largely upon differences in the measurements as influenced by the individual variation in the degree of the body curve.

Plate 1 shows the right side of a corrosion reconstruction of the pharynx and arterial arches in a 6-mm. cat embryo (C.U.E.C., series 889) and represents the earliest stage in the accompanying series of four models here selected as illustrations. The branchial pouches I to V are indicated fully, and IV is differentiated clearly from V by a deep narrow groove. The pulmonary anlage consists of a short blunt ventral evagination in wide communication with the oesophagus, its caudal termination showing a mere indication of bifurcation.

The first and second aortic arches are already discontinuous and broken up into their cranial terminals. The third and fourth arches are complete, the former being the larger of the two. On the fourth arch the point of confluence of its ventral and dorsal anlagen is still indicated by a narrowed isthmus and an irregular ventrocranially directed spur.

The formation of the rudiments of a fifth arch (5, 5) and the development of the sixth arch (6, 6) are still in their inception. The ventral anlage of the fifth is represented by a short blunt projection from the caudal border of the fourth arch, just ventral to the fourth pouch. The dorsal element of the fifth appears as a similar somewhat smaller protrusion from the dorsal aorta, caudal to its connection with the fourth arch. The ventral and dorsal beginnings of the sixth arch are clear. The former appears as a short rounded process arising from the caudal margin of the fourth arch, midway between the ventral rudiment of the fifth arch and the truncus.

The dorsal rudiment of the sixth arch arises from the dorsal aorta, extending cranial as a short spur. This I shall designate as the cranial member in a series of aortic derivatives which, inosculating in a capillary network, form what may be defined as the postbranchial pulmonary plexus, in view of the part it takes in the development of the pulmonary artery, as described in detail below. The succeeding two components (7, 8) follow

in close proximity to 6, and the three branches form together a broad aortic connection, pierced by two rounded foramina. From this a network of capillaries extends ventrad into the tracheogular area. The ninth aortic derivative enters the plexus considerably further caudad opposite to the pulmonary evagination.

Plate 2 shows the right side of the same region in another 6-mm. embryo (C.U.E.C., series 885), in which the reconstruction has followed the ectal surface of the pharyngeal epithelium. The IVth pouch has enlarged, especially in its sagittal dimension, and the Vth appears more distinctly as its caudal prolongation, owing to the broadening out and shallowing of the deep narrow sulcus separating the IVth and Vth evaginations in the preceding embryo (series 889, pl. 1).

The first and second aortic arches are, as before, interrupted, the third and fourth are continuous, and the sixth has become established as the completed pulmonic arch.

Compared with the preceding stage (pl. 1), the area caudal to the fourth arch has undergone a considerable and significant rearrangement, owing primarily to the increased surface of the pharyngeal wall corresponding to the IVth and Vth evaginations, and to the completion of the sixth aortic arch.

The ventral extremities of the aortic arches have joined in an extensive bulbus which is definitely set off against the truncus. The sixth arch arises on each side as a continuation of the caudolateral angle of the bulbus, curves caudodorsad to pass on the medial surface of the Vth pouch, and enters the dorsal aorta by a wide, irregularly expanded terminal which develops from the cranial end of the postbranchial pulmonary plexus of the preceding stage. With the completion of the sixth arch and the enlargement of the pharyngeal area IV to V, the fourth arch moves further craniad relative to the pharynx as a whole. It is lodged in the depth of the third branchial groove and overlapped ectally by the tip of the third pouch.

The rudiments of the fifth arch now appear (pl. 2, 5, 5) in the space occupied by the IVth and Vth evaginations and framed by the fourth and sixth arches, the corresponding portions of

the bulbus and the dorsal aorta. The dorsal anlage of the fifth arch is seen as a short spur arising from the dorsal aorta between 4 and 6. The ventral rudiment of the fifth arch forms a somewhat longer projection from the beginning of the ventral segment of the pulmonic arch. Both the dorsal and ventral fifth arch anlagen occupy exactly the same horizontal level, corresponding to the groove between the IVth and Vth branchial pouches.

The ventral protons of the fifth and sixth arches, which are seen in series 889 connected with the caudal border of the fourth arch (pl. 1, 4, 5), have become joined in series 885 and separated from the fourth arch, the extension caudad of the sixth arch carrying the ventral anlage of the fifth arch with it into the position and relations shown in plate 2. The pulmonary development in embryo 885 is relatively far advanced. The tracheal tube has separated from the oesophagus in its distal third, and the larger right lung already shows the beginning budding of the eparterial, cardiac, and first and second ventral hyparterial bronchi. The postbranchial pulmonary plexus has extended ventrad into the pulmonary area and is closely associated with the cranial portion of the developing lung, while a number of isolated vascular islands indicate the line of its future extension into the caudal districts. The dorsal aortic tributaries to the plexus are indicated in the reconstruction, numbered 7 to 12. The cranial end of the plexus is still connected with the dorsal aortic root of the sixth arch by the same broad capillary confluence observed in the preceding embryo 889, and the same intervals between 6, 7, and 8 are retained as wider meshes of the reticulum. Aortic branch 10 is of notable length and caliber, and reaches the plexus near the commencement of its distal third. Branches 9, 11, and 12 are connected with the plexus through delicate capillary anastomoses not reproduced in the model.

In comparing the two models shown in plates 1 and 2, the following changes are significant:

1. The increase in the longitudinal measure of the lateral pharyngeal wall carrying the IVth and Vth evaginations has approximated them more closely to the cranial elements of the postbranchial plexus, and hence to the dorsal anlage of the sixth arch.

2. At the same time the ventral anlage of the sixth aortic arch, carrying the ventral rudiment of the fifth arch with it, has moved caudad, opening up an interval between it and the fourth arch in which the two rudiments of the fifth arch are brought to face each other at the level of the ectal groove separating the IVth and Vth pouches.

3. The sixth arch has thus acquired an independent ventral origin from the aortic bulbus.

4. This shift and the accompanying further development has brought the cranial end of the postbranchial plexus, carrying the dorsal anlage of the sixth aortic arch, nearer to the dorsal end of the fourth and the dorsal rudiment of the fifth arch, while the corresponding ventral interval has been lengthened by the separation of the fourth and sixth arches at the bulbus and the downward extension of the latter vessel. These shifts of the dorsal and ventral pulmonic anlagen balance each other and thus bring the two anlagen to the same level in the angle formed between the last pharyngeal evagination and the lateral wall of the postbranchial intestine.

5. The sixth aortic arch differs, therefore, from the preceding arches in the mode of development of its ventral and dorsal anlagen. While the former shares with the remaining aortic arches an ultimate origin from the aortic bulbus, the latter is from the beginning part of a plexus organized in direct relation to the developing lung, from which it separates only secondarily to furnish the material for the establishment of the Botallian duct.

The important facts in the development of the complete pulmonary artery in relation both to the sixth arch and to the post-branchial pulmonary plexus will be considered after the remaining stages here selected for illustration have been described.

Plate 3 shows the right side of a lumen reconstruction of the pharynx and aortic arches in a 4-mm. cat embryo (C.U.E.C., series 771). The first and second arterial arches are interrupted, the third and fourth complete. There is no trace of a ventral rudiment of a fifth arch, derived either from the fourth or from

the sixth arch. But a well-developed dorsal anlage of the fifth arch arises from the aorta, and is lodged in the distinct transverse sulcus separating the IVth and Vth pouches. Abutting against the blind ventral termination of the rudimentary fifth arch, between it and the ventral pulmonic anlage, is an isolated arterial island, which might, in the course of further development, either form a continuation of the fifth arch ventrad or join the ventral element of the sixth arch under cover of the Vth evagination in completing the pulmonic arch. In view of the course of development of the pulmonary artery in a number of embryos examined, I am inclined to accept the latter interpretation of the isolated vascular island in question for reasons stated in detail below. (cf. pp. 174, 175.)

The sixth arch is outlined clearly by its dorsal aortic and ventral bulbar components, which have not yet fused caudomesal to the Vth pouch. The dorsal anlage of the pulmonic arch again arises from the dorsal aorta in continuity with the cranial end of the postbranchial pulmonary plexus. The foramen between dorsal 6 and 7, previously noted in series 889 and 885, appears somewhat elongated. The plexus further caudad receives four additional aortic branches (8 to 11) which inosculate on the walls of the intestinal tube and developing lung. The latter corresponds to the stage shown in series 885. The caudal segment and bifurcation of the trachea are free from the oesophagus. The cast of the right pulmonary lumen shows the beginnings of the eparterial, cardiac, first and second ventral hyparterial buds. The ascending bud from the first ventral hyparterial appears on the tree of the left lung. The ventral proton of the sixth arch arises from the right caudal angle of the bulb, close to its connection with the fourth arch, and descends along the entire ventral margin of the IVth and Vth pouches to end in a blunt recurved hook caudal to the above-described isolated arterial anlage.

Finally plates 4 and 5 show the right and left views of a reconstruction of the same region in another 4-mm. cat embryo (C.U.E.C., series 773), in which the model follows the ectal line of the intestinal epithelium.

1. Plate 4. Right side of model 773. The fourth arch is complete, but is partly reduplicated in its dorsal portion. I am inclined to assign to the smaller caudal element completing the foramen the tentative value of a rudimentary and incomplete fifth arch. This interpretation is in part supported by the conditions illustrated in embryo 889, in which pouches IV and V appear as distinct pharyngeal evaginations separated by a sharp narrow sulcus (pl. 1). If the arterial spurs 5 to 5 were joined, connecting the fourth arch with the dorsal aorta, the resulting fifth arch would occupy this groove between the IVth and Vth pouches, and the theoretical postulates for a fifth branchial arch and artery would be met.

In embryo 773 (pl. 4) the IVth and Vth pouches are practically confluent on both sides, and a fifth branchial arch is wanting. The dorsal rudiment of the fifth aortic arch may be considered to have moved cephalad over the smooth ectal surface of the last pharyngeal evagination, instead of lodging in a groove between the IVth and Vth pouches, and to have become partially submerged in the fourth aortic arch, forming the caudal boundary of its foramen. Ventral to this a blunt spur from the fourth arch (not labeled in the plate) agrees with the ventral rudiment of the fifth arch (5) found on the right side of embryo 889 (pl. 1).

The elements of a right fifth arch in series 773 appear to approach the conditions described by Zimmerman (2) in man. They also resemble the vessel described and figured in a 6.5-mm. pig embryo by Reagan (16, p. 497, fig. 15), who considers it, as well as Zimmerman's case, merely as division of the fourth arch.

The right sixth arch of series 773 is not yet completed. The shorter dorsal component arises from the dorsal aorta by a broadly expanded root, indicating the incorporation of some of the cranial elements of the postbranchial plexus, as in the preceding instances. The ventral anlage of the sixth arch descends from the bulb as a long vessel (cf. series 771, pl. 3), which, as it turns dorsad under cover of the Vth pouch, sends a blunt cone-shaped spur caudad toward the postbranchial plexus. In contrast to the preceding instances, the plexus has lost its direct

connection with the dorsal root of the sixth arch. It is now supplied by four caudal aortic branches (7 to 10), which inosculate on the dorsal and lateral walls of the intestinal canal and lung. The latter is approximately in the same stage of development as series 771 (pl. 3).

2. Plate 5. Left side of model 773. The second aortic arch is still continuous. The IVth and Vth branchial pouches are practically merged, as on the right, into a single evagination. The rudiment of the fifth arch is represented by two short spurs arising from the dorsal aorta in the interval between the fourth and sixth arches. There is no corresponding ventral fifth anlage. The two sides of the reconstruction suggest that the practical confluence of the IVth and Vth pouches, and the consequent elimination of the fifth branchial arch, are factors bearing on the extreme reduction of the fifth aortic arch.

I can completely endorse the opinion expressed by Coulter (13, p. 590). "The facts observed point to the conclusion that ordinarily no fifth aortic arch is completely developed in the cat, and it seems more than probable that the incomplete development and uncertain character of the fifth aortic arch is merely an expression of the incomplete development of the fifth branchial arch." The sixth aortic arch has been completed by junction of its ventral and dorsal components under cover of the last pharyngeal evagination. The dorsal element, forming the anlage of the ductus arteriosus, has a double connection (6 and 7) with the aorta. The caudally directed spur of the ventral anlage, noted on the right arch (pl. 4), has on the left side effected a junction with the cranial and ventral portion of the postbranchial plexus, and serves now as the main channel carrying the blood from the ventral segment of the pulmonic arch to the foregut and developing lung. In conformity with this rearrangement the postbranchial plexus shifts further ventrad to the dorso-lateral aspect of the lung. It begins to lose its multilocular character of a capillary plexus with the development of a larger central channel, now in direct continuity with the ventral segment of the sixth arch, both together constituting the bed for the development of the definite pulmonary artery.

The plexus still has numerous connections (8 to 14) with the dorsal aorta, caudal to the Botallian duct (6, 7), but they appear reduced in caliber, as if drawn out by the shift ventrad of the main channel into the line of the caudal continuation of the ventral pulmonic arch. The plexus as a whole is in the process of abandoning its primary multiple aortic connections and of transferring to the new line of the future pulmonary artery, initiated by its junction with the caudal spur from the ventral segment of the sixth arch.

GENERAL CONSIDERATIONS AND SUMMARY

1. *Morphogenesis*

It appears, on the evidence presented by the earlier stages in the cat, that the mammalian pulmonary arteries do not arise primarily as buds or outgrowths from the ventral segments of the two sixth aortic arches, and that they do not attain their destination by a process of continuous growth caudad, but that they develop by the concrescence of a number of originally independent vascular anlagen, which obtain a secondary connection with the sixth arch of each side, and thus lead to the establishment of the definite condition.

Caudal to the last pharyngeal evagination, the early perigular vascular spaces organize into a series of irregular channels opening into the dorsal aortae, which then appear to give origin, fairly symmetrically on the two sides, to from four to eight horizontal ventromedial branches directed toward the post-pharyngeal intestinal tube, on which they form an inosculating capillary reticulum. With the development of the pulmonary anlage, this meshwork occupies the oesophageotracheal gutter and its distal elements spread over the lung-buds, forming a primitive pulmogular plexus. While this is organizing, the sixth aortic arch is laid down caudal to the last pharyngeal evagination. It develops, as do the remaining aortic arches, by anlagen which connect early with the dorsal and ventral aortae and appear hence as outgrowths or buds derived from them. These become joined to vascular islands developed independently along the

line of the branchial arch. The ventral pulmonic anlage appears first as a blunt projection attached to the ventral extremity of the fourth aortic arch, close to its connection with the aortic bulb, to which its origin becomes subsequently transferred. It extends, partly by its own growth, partly by the incorporation of locally formed vascular spaces lying in its path, caudodorsad, along the ventral and caudal border of the last pharyngeal evagination, finally coming to occupy the mesal surface of the Vth pouch. The dorsal anlage of the sixth aortic arch arises from the dorsal aorta and constitutes at first the cranial component of the pulmogular plexus, gaining a greater independence from the latter as the sixth aortic arch becomes defined, and the plexus caudal to this point passes ventrad into more intimate relation with the developing lung. The dorsal and ventral anlagen of the pulmonic arch gradually approach each other in the manner described and join under cover of the last branchial protrusion to form the completed channel of the sixth arch. The method by which this junction is finally accomplished constitutes one of the most important steps in the development of the pulmonary artery. The two anlagen do not meet in an end to end fusion, but the dorsal component joins the longer ventral channel some distance above the latter's blind termination. The result is that, when the sixth arch is completed, its ventral segment is provided with what appears as a caecal outgrowth, directed caudad, toward the cranial end of the postbranchial pulmonary plexus. In the interim the latter has formed its main longitudinal channel, the proton of the future pulmonary artery, which becomes completely established by junction with the blunt ventral appendage of the sixth arch.

The apparent 'outgrowth' from the sixth arch, described in the current accounts as leading to the development of the pulmonary artery by a continued extension caudad, is therefore nothing but the blind end of the original ventral anlage of the sixth arch, left as an appendage when the arch is completed by meeting the extremity of the dorsal anlage in the manner above described. The main pulmonary artery develops independently of the sixth arch by the organization of a distinct arterial channel

in the ventral portion of the postbranchial pulmonary plexus. The so-called 'outgrowth' from the sixth arch serves merely as the point of junction at which, after coalescence with the pulmonary plexus, the blood is carried from the ventral segment of the sixth arch into this prepared channel of the pulmonary artery. The 'outgrowth' would be more correctly defined as the pulmonary arterial 'tap' or 'approach' of the sixth arch.

This ontogenetic history of the sixth arch in its relation to the development of the pulmonary artery is uniformly encountered in embryos of the cat between 4 mm. and 6 mm. crown-rump measure. The steps involved can be followed in the preparations selected to illustrate this paper, and are indicated schematically in the accompanying diagrams (text figs. 1 and 2).

Plate 1 (series 889) shows the initial stages in the development of the sixth arch. The ventral anlage (6) appears as a blunt protuberance incorporated in the ventral end of the fourth arch. The dorsal anlage (6) arises from the dorsal aorta in direct connection with the cranial end of the primitive pulmogular plexus, which occupies the dorsolateral wall of the postpharyngeal intestine and hardly extends as yet to the pulmonary bud.

In plate 3 (series 771) the longer ventral and shorter dorsal components of the sixth arch have formed and approach each other, encircling the Vth pouch. As previously stated, I regard the isolated arterial space, situated between the end of the rudimentary fifth arch and the ventral anlage of the sixth, as destined to be incorporated in the latter, and to furnish the link which, in further development, would have completed the arch by concrescence with the dorsal anlage under cover of the Vth pouch. If this had occurred, the actual blind end of the ventral anlage of the sixth arch (to which the short dotted leader runs in the plate) would be left as an appendix projecting from the sixth arch caudad toward the cranial end of the postbranchial plexus.

In plate 4 (series 773, right side) the ventral anlage of the sixth aortic arch is again very long, the dorsal short. It may be fairly assumed that the former, by parasynaptic accretion of a vascular island similar to the isolated space shown in plate

3, has been carried further dorsad along the mesal side of the last branchial evagination, leaving its original terminal as the blind appendage seen extending caudad.

Plate 2 (series 885) shows the condition which completion of the sixth aortic arch would have produced in the preceding

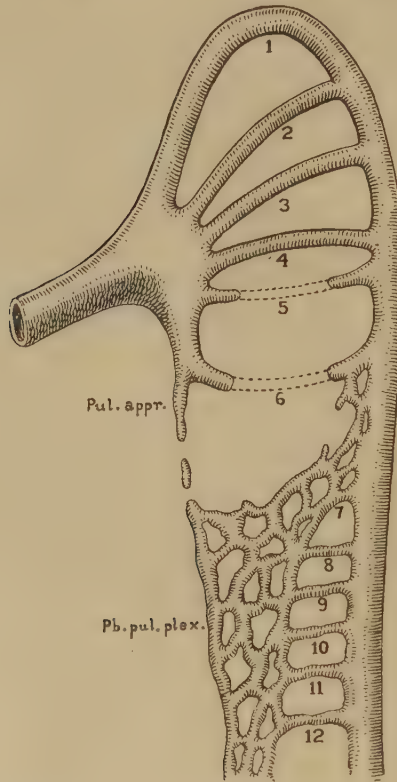


FIGURE 1.

Figs. 1 and 2 Schemata of the development of the aortic arches, postbranchial pulmonary plexus and pulmonary artery. Fig. 1, early stage; fig. 2, later stage.

embryo, leaving the blind appendix attached to the ventral segment of the arch.

Finally, in plate 5 (series 773, left side) the junction of this appendix with the cranial end of the postbranchial pulmonary plexus has occurred, and the definite permanent path of the pulmonary artery has been established, leading from the heart via the sixth arch to the lung.

The successive changes which take place in the postbranchial pulmonary plexus involve:

1. A gradual shift of the plexus ventrad into closer proximity to the developing lung. Plates 1, 5, 4, 3, 2, show the stages in this process, in the order named.

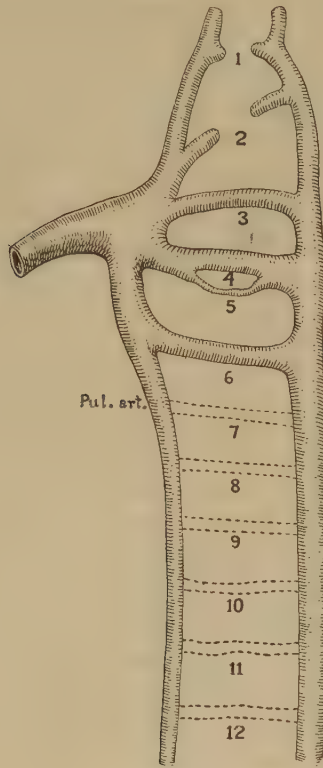


FIGURE 2.

1 to 6, aortic arches; 7 to 12, dorsal aortic branches. *Pb. pul. plex.*, postbranchial pulmonary plexus; *Pul. appr.*, pulmonary approach of sixth aortic arch; *Pul. art.*, pulmonary artery.

2. This change from a dorsal to a ventral parapulmonary plexus is effected partly by a lengthening of its primitive connections with the dorsal aorta, partly by the interruption of a number of them (pls. 2, 3).

3. The establishment of a larger, better-defined channel within the ventral segment of the plexus, which is to serve as the fundament of the main pulmonary artery.

4. The gradual separation of the dorsal anlage of the sixth arch from the cranial extremity of the postbranchial pulmonary plexus, leaving the former as the basis for the Botallian duct (pl. 4).

5. The junction of the cranial extremity of the postbranchial pulmonary plexus with the above-described appendage to the ventral segment of the sixth arch, thus establishing the definite path of the pulmonary artery (pl. 5).

The origin of the pulmonary artery shifts, therefore, ontogenetically from the dorsal aorta first to the rudiment of the Botallian duct and then to the ventral segment of the sixth arch, simulating an 'outgrowth' from the latter, but actually signaling the final step in the concrescence of the branchial and postbranchial elements entering into the composition of the adult vessel. This is illustrated by the following series in the order named: plates 1, 3, 2, 4, 5.

The above-described phases in the ontogenetic history of the mammalian pulmonary artery, as illustrated in the cat, occupy a relatively short period in which they do not follow each other in a strict chronological order which is uniform for every embryo. In any individual one or another of the events comprised in the early development cycle of the pulmonary artery may make a precocious appearance or be delayed. Hence the examples here used as illustrations of each phase do not maintain their exact numerical order throughout the entire period, but are intended to cover all the events of the early stage as a whole. For example, in plate 5, closing the series, the postbranchial pulmonary plexus is still connected at numerous points with the dorsal aorta, although the anlage of the pulmonary artery has effected its junction with the ventral part of the sixth arch, while on the other hand, in plate 2 the separation of the postbranchial plexus from the aorta is far advanced, although the connection with the sixth arch is not yet established. The early period thus includes the completion of the sixth aortic arch, the formation of its ventral "outgrowth," more properly defined as its 'pulmonary approach,' and the organization of

the postbranchial pulmonary plexus developing concurrently as a whole, but with slight chronological variations in the appearance of these events in individual embryos.

2. Phylogenetic considerations

In their phyletic significance the early ontogenetic stages of the mammalian pulmonary artery place this vessel in direct serial line with the respiratory vascular channels of the lower vertebrates and outline the common vertebrate ground-plan for this portion of the haemal vascular system. The establishment of a respiratory apparatus, other than the cutaneous exchange of the blood gases, depends upon the morphological adaptations to this purpose of shorter or longer areas of the intestinal entoderm, whatever form the resulting structure may finally obtain in the different vertebrate classes. Modifications of the vascular system, adapting this entodermal respiratory derivative to its functional purpose, develop in correlation with the morphogenesis of the respiratory organ in response to the varying environmental and metabolic demands of the vertebrate types. The proximity and parallel course of the intestinal tube and aorta in the dorsal axial line naturally bring both structures into coöperation in the development of the respiratory tract, and branches of the dorsal aortae function from the beginning as the afferent respiratory channels. The concept is justified that in the prevertebrate and in the earliest vertebrate types the exchange of the gases of the body was carried on by a series of modified epithelial areas of the intestinal tube, perhaps extending over the greater part of the entire canal, and supplied by a corresponding metameric series of intersegmental afferent vessels from aorta. The numerous gill-slits of *Amphioxus* and the multiple internal gill-pouches of *Cyclostomes* (*Bdellostoma*, *Myxine*) support this assumption among extant forms. The next step in vertebrate respiratory organization is, of course, based on the more favorable location of the cranial portion of the intestinal canal for the intake of the aerating water through

the oral opening, and on the advantage offered by a reduced number of larger afferent vessels arising in close proximity to the heart. These two factors resulted in the specialization of the pharynx and the development of the branchial apparatus. With the acquisition and further elaboration of this highly concentrated respiratory organ, the series of the aortic arches develop as the sole afferent vessels in the higher water-breathing types, and the more caudally situated primitive multiple aortic afferents are suppressed or diverted to other functions of the digestive tract. With the final replacement of the branchial by the pulmonary type of respiration a rearrangement of the vascular relations is necessitated. This calls into service both the distal aortic arches and their connection with the truncus of the heart, and again resuscitates the series of the primitive aortic afferents supplying in the early phyletic stages the respiratory areas of that portion of the intestinal tube which now, as its postbranchial segment, forms the site of the final pulmonary evagination. In tracing this brief outline of the phylogenetic history of the pulmonary artery in the higher vertebrates, it is significant to note that in the perennibranchiate Amphibians, possessing both gills and primitive lungs, some concrete evidence is to be found in support of the view here expressed. Thus, Miller (5 a) describes the pulmonary artery in *Necturus* as arising from the combined channel of the efferent second and third aortic arches and sending small branches to the muscles of the pectoral region on its way to the lung. Williams (14) compares these pectoral branches to the large cutaneous branches of the pulmonary artery found in the frog. They might equally well be interpreted as primitive respiratory aortic branches which, with the development of the branchial apparatus, were diverted to the somatic supply, but which the appearance of the lung has in part swept into the newly organized pulmonary artery, in accordance with the ontogeny of this vessel outlined above. A definite answer as to the significance of the pulmopectoral branches of *Necturus* and of similar vessels in other forms calls for the detailed study of the early ontogeny of their pulmonary artery.

Further, Williams (l.c., pp. 409-413) describes and figures instances in *Necturus* in which a lung is either entirely supplied by a caudocranially directed branch of the seventh intercostal artery, with default of the typical pulmonary artery, or receives pulmonary branches from the dorsal aorta supplementing a reduced pulmonary artery derived from the efferent branchials. Accessory pulmonary arteries arising from the coeliac, or from the mesenteric arteries have also been recorded in the frog by Mudge (11) and Warren (3).

In several Lacertilian reptiles (*Iguana*, *Cyclura*) the lung receives normally one or more accessory pulmonary arteries from the aorta or from the mesenteric arteries, and the adult arrangement of the entire pulmonary arterial supply corresponds strikingly to the above-described mammalian stages in embryos of the cat.

Hyrtl describes similar constant accessory pulmonary arteries in the *Ophidia* arising from the aorta or from some of its splanchnic branches (oesophageal, gastric, hepatic). These scattered and rather incidentally recorded observations in the lower vertebrates could unquestionably be greatly multiplied by a systematic investigation covering a wider range of material and types. They constitute, in my opinion, a group of very important facts bearing directly on the phyletic path followed by the mammalian lung and its vessels, and closely linked with the ontogenetic history of the pulmonary artery as outlined above. I find myself obliged to differ emphatically from the opinion expressed by Williams in summing up his observations on *Necturus* (l.c., p. 414) when he says: "The results of further investigation indicate that the occurrence of pulmonary branches of the aorta in the various classes of vertebrates has an embryological rather than an evolutionary significance. The connective-tissue pathway being provided, capillary branches from the adjacent vessels will enter it, and occasionally, as in these *Necturus* specimens, certain of them will become very large, and notable as anomalies." This view, in my judgment, deliberately throws overboard the only rational ontogenetic and comparative anatomical guiding line which we possess for the sound interpretation

of reversional phylogenetic variation. It is true that the vertebrate organization is provided throughout with abundant 'connective-tissue pathways,' and the primitive dorsal mesentery of the intestine is among the most archeal of these structures. The entire vertebrate vascular system owes its origin to the inherent potency of the mesenchyme to differentiate locally all the components of the vascular anlage. A developing channel does not simply 'enter' a 'connective-tissue pathway' serving merely passively as the road along which an extension from preexisting adjacent vessels travels carrying a structure of foreign derivation into new territory. The 'pathway' itself furnishes the material for the construction of the new vessel on the spot by mesenchymal differentiation. This does not occur by chance or at random, nor because a 'pathway' happens to be available, in a place where the entire territory is potentially 'pathway' in the sense of providing the cellular basis for the development of a vessel, if the proper impulse for such development exists. This determinant is offered by the definite fundamental ground-plan underlying all vertebrate angiogenesis, however greatly it may become modified with the branching of the parent stem.

Certainly, a set of vessels which develop uniformly during the mammalian ontogeny, which constitute part of the normal vascular system in some of the lower vertebrates, which appear as variants in individuals of different vertebrate classes, and which, moreover, fit into a common genetic plan of vertebrate organization, are not the outcome of a haphazard accidental wandering of their components into strange and unusual 'connective-tissue paths'. They develop in strict conformity to the impulse derived from the entire ancestral background of the individual which we define as Heritage and recognize as the recording angel of evolution.

To return to the case here under discussion, certain human variations of the pulmonary arteries, in addition to those of the bronchial arteries, merit consideration in this connection and find their explanation in the embryonic history of these vessels. To this group belong the following, recorded mostly by Krause (19), Schwalbe (18), and Förster (17):

1. Origin of the left pulmonary artery from the left subclavian, or anastomoses between the two vessels.
2. Origin of the right pulmonary artery from the innominate trunk.
3. The right subclavia arising from the bifurcation of the pulmonary trunk.
4. The left subclavia forming the continuation of the Botallian duct.
5. Of special significance are the cases collected by Peacock (20), in which the aortic arch defaults or is interrupted and the descending aorta forms a continuation of the pulmonary artery. Herxheimer (18, p. 461) describes and figures a case in which the aorta gave origin to the innominate, left carotid, and left subclavian arteries and then ended blindly. The pulmonary artery continued directly through an enlarged Botallian duct into the descending aorta, supplying intercostal and abdominal branches, and dividing into the common iliac vessels.

The origin and variations of the bronchial arteries, and the presence of anastomoses or larger connecting channels between them and the pulmonary arteries, likewise depend on the organization of the early postbranchial pulmonary plexus.

Finally, the ontogenetic facts here recorded have an important bearing on the phylogenetic interpretation of the intrapulmonary architecture of those mammalian types in which the prevalent dorsolateral relation of the main pulmonary artery to the stem-bronchus and its primary derivatives is exchanged for a course in which the vessel passes ventral to one or more of the primary ventral bronchi. This is the normal relation of the structures in the right lung of *Alouatta seniculus* and on both sides in *Sphingurus prehensilis*. It is recorded as a more or less frequent variation in individuals of a number of mammalian genera:

Primates:

Man.

Macacus erythraeus.

Macacus nemestrinus.

Cercocebus sinicus.

Hapale rosaria.

Insectivores:

- Pteropus edulis.
- Vesperugo pipistrellus.
- Erinaceus europaeus.

Carnivores:

- Phoca vitulina.
- Felis lynx.

Ungulates:

- Catoblepas gnu.
- Capra ibex.
- Bos taurus.

Edentates:

- Dasypus novemcinctus.
- Dasypus setosus.

Mono remes:

- Echidna aculeata.

The existence of these atypical relations of the pulmonary artery to the ventrolateral derivatives of the stembronchus point clearly to the potential development in the mammal of a secondary ventral arterial channel, supplementary to the main dorso-lateral pulmonary artery, and capable of partly or wholly replacing the same, either regularly in a few species (*Alouatta*, *Sphingurus*), or as an individual ontogenetic variation in a more extended range of mammalian types. The evidence now afforded by the organization of the postbranchial pulmonary plexus demonstrates the potential development of intrapulmonary arterial lines both ventral and dorsal to the axis of the early pulmonary cavum which constitutes the basis of the future stembronchus and its primary branches.

While the dorsal arterial path becomes in the vast majority of mammalian types the typical and more constant channel, following the dorsolateral circumference of the stembronchus and intersecting its primary derivatives on their dorsal aspect, the morphogenetic history of the mammalian pulmonary artery clears the field of hypothetical considerations by furnishing a plan of intrapulmonary arterial development according to which either the dorsal or ventral line is able to develop, either exclusively or predominately.

BIBLIOGRAPHY

Since the appearance of Zinmermann's (2) pioneer publication in 1889, the site of the developing pulmonary artery in the mammalian embryo has been studied in detail by a number of investigators owing to the interest aroused by the question of the fifth aortic arch and the ensuing discussion regarding the nature of the post- or ultimobranhial pharyngeal evagination. It is, as stated, generally assumed that the mammalian pulmonary artery develops as a bud or outgrowth from the ventral segment of the sixth aortic arch.

His (1 a) in 1885 refers briefly to the development of the pulmonary arteries as arising early from the caudal aortic arch of each side, in human embryos of between 4.2 mm. and 5 mm. total length (fig. 121 of text; Taf. I^x. figs. 4 and 5 of Atlas). In 1887 he published (1 b) the first detailed account of the development of the lung in the human embryo, in which he describes the appearance of the anlagen of the pulmonary arteries as about coinciding with the completion of the last aortic arches, arising from these at the level of the future arytenoids and descending steeply along the ventral border of the tracheal anlage. He concludes his account of the early ontogenetic period of these vessels with the statement (p. 102), "Über das Verhalten des untersten Gefässendes während der frühen Entwicklungsstufen vermag ich keine entscheidenden Angaben zu machen."

His' account has been handed down practically unchanged, except for the numerical value assigned to the pulmonic arch in the series of the aortic arches, and forms the basis for the modern genetic interpretation of the vessel.

Narath, who published an extensive monograph on the comparative anatomy of the mammalian lung (4) in 1901, devotes a special paragraph (i.e., pp. 283, 284) to the development of the pulmonary artery in the rabbit. He follows the stereotyped account, describing the vessel as arising in embryos of the eleventh day from the lowest point of the ventral segment of the sixth aortic arch and descending along the pulmonary anlage at first in complete symmetry with the same. He does not refer to the

existence of any contributions from the dorsal aortae. He gives a somewhat schematic figure (tab. I, fig. 2) of a cleared preparation of a $7\frac{2}{3}$ mm. rabbit embryo, showing the relation of the artery to the trachea and lung-bud. There is no reference to any other source of origin for the pulmonary artery.

Bremer, in his two earlier publications (8 a, 8 b), accepts the pulmonary arteries as continuous symmetrical outgrowths, one from each pulmonary arch. The two papers deal mainly with the mechanical aspects of the main pulmonary truncus in its torsion about the aortic bulb, around which the sixth arches are wound, and the resulting location of their points of connection with the proximal artery. He describes the proximal fusion of the two pulmonic arches by absorption of their walls brought into contact by the twist, thus lengthening the truncus at their expense and moving the point of bifurcation continually farther from the heart. In addition to this fusion of the ventral segments of the sixth arches, Bremer describes the approximation of the pulmonary arteries and the development of an anastomosis between them. He also speaks of the pulmonary arteries as developing in a capillary network. In his third paper (8 c) Bremer describes the pulmonary arteries in embryos of the rabbit as arising, not from the pulmonary arches, but from a ventral aortic net, which extends beyond the arch. He says (l. c. p. 126): “. . . . this extension of the ventral aortic net forms well defined pulmonary arteries, one on each side before the pulmonary arch exists; the pulmonary artery is in no sense a branch of the pulmonary arch, and moreover, in the strictest sense, the arch extends only from the dorsal aorta to the pulmonary artery, the ventral part of the vessel usually called the arch is really the ventral aorta. The persistent pulmonary arteries are entirely ventral; they have been joined during embryonic life by branches from the dorsal aorta, but such branches are only temporary.”

Bremer sums up his conclusions (l. c. p. 127) as follows:

A further extension of the plexus of the ventral aorta, situated between the floor of the pharynx and the dorsal wall of the pericardial cavity, but prevented from crossing the median line by the presence

of the median pharyngeal outgrowth to form the trachea, extends to the lungs as the pulmonary arteries, which are later joined by vessels springing from the dorsal aortae. These vessels, which may be double and plexiform, constitute the fifth (and sixth) arch.

These highly interesting observations of Dr. Bremer's on rabbit embryos do not agree with my findings in cat and rabbit embryos, as described in the body of this paper. On the evidence furnished, at least by my material, I am obliged to ascribe real morphological value to the ventral anlage of the sixth arch, which cannot be merged into a caudal extension of the ventral aorta.

As shown in the above-described series of cat embryos, the sixth arch is either well on its way to completion (pls. 3 and 4) or actually has joined the dorsal anlage before the pulmonary artery, still included within the postbranchial plexus, has united with the process from the sixth arch. This union (pl. 5) I must regard as the final step in the establishment of the definite path of the persistent pulmonary artery.

As regards the contributions from the dorsal aortae, Bremer regards them as forming the fifth and sixth arches, which do not arise from the ventral aortic net.

In a subsequent communication (8 d) Bremer quotes a short article published in the Russian language by Fedorow in the "Communications of the Military Med. Akad." St. Petersburg, vol. 22, no. 1, 1911, describing the origin of the pulmonary arteries in the guinea-pig. As appears in the translation furnished very kindly by Dr. Bremer of extracts from this inaccessible paper, Fedorow describes the pulmonary arteries as arising in embryos of the eighteenth day (30 somites) from the fourth aortic arches of each side.

They begin near the truncus arteriosus ventral to the middle part of the pharynx, which is quite large at this point. The arteries run caudally on the ventral surface of the oesophagus, dorsal to the pericardial cavity, and quite near to each other. Further down, the oesophagus appears compressed laterally, and the arteries lie lateral to its ventral part, which projects in the form of a pointed keel. The pulmonary arteries end blindly, traversing about 20 segments (240 μ). In another embryo of the same age, with 29 somites, the arteries extend

further, their diameter varying at different points, and it may be anastomose with the vessels of the oesophagus. Similar anastomoses frequently occur later. One notices the double origin of both arteries from their corresponding arches, and they may even join one another near the arch. Island formation occurs along the course of the arteries.

In the embryo of the nineteenth day, with 32 somites, the third and fourth pairs of aortic arches are present, the sixth pair represented by blind growths both from the dorsal aortae and from the truncus arteriosus. The delicate pulmonary arteries begin from the sixth pair as short growths. Properly, they are the elongated aa. pulmonales of the earlier stages. They run caudally ventrolateral to the oesophagus, and anastomose with its vessels.

Fedorow's description of the first anlagen of the pulmonary arteries arising in the guinea-pig from the "medial wall of the fourth arches, near the truncus arteriosus," corresponds entirely with the condition observed in the cat (cf. pl. I, 6, pp. 168-169), prior to the development of a separate point of origin from the extended aortic bulbus, as the now independent ventral anlage of the sixth arch. I take it that this is what the Russian observer implies when he describes the pulmonary arteries of his 32-somite embryo as arising from the sixth arches as small growths: "Properly, they are the elongated a.a. pulmonales of the earlier stages."

Fedorow speaks of anastomoses between the pulmonary arteries and the oesophageal vessels, but does not assign to the latter any share in the formation of the definitive pulmonary artery, which remains an outgrowth from the aortic arches.

Coulter (13, p. 587), describing a 5.6-mm. cat embryo (series 110 of the Columbia University Collection), says: "Each pulmonary arch joins the dorsal aorta by three distinct roots, not clearly shown in the figure." This evidently refers to the cranial portion of the postbranchial plexus and its connection with the dorsal anlage of the sixth arch, which, as above described, is primarily a plexus derivative. Further in the same paper (p. 587), in describing a 7-mm. embryo (series 138, C. U. E. C.), he finds that "the dorsal end of the sixth aortic arch is very large, and on the right side shows a peculiar grooving which is suggestive of a division into two much longer roots than found elsewhere."

Finally (p. 590) Coulter concludes: "It may well be that the anastomoses and irregular roots about the base of the pulmonic arch which have been so generally described in the mammalia are evidence of an assimilation of the fifth aortic arch into the pulmonic, beginning at their dorsal extremities. . . . The dorsal root of the pulmonic arch in every case, from its first appearance until after the buds of the pulmonary arteries arise, was found to be pierced by two or more 'islands.' The significance of this has been referred to above."

These are the only passages which I can find in the literature indicating that at least the cranial extremity of the postbranchial pulmonary plexus has been observed and noted in connection with the dorsal anlage of the sixth arch, although its entire organization and significance escaped recognition.

- 1 HIS, WM. 1885-1887. (a) Anatomie menschlichen Embryonen. III, S. 186. 1885.
(b) Zur Bildungsgeschichte der Lungen beim menschlichen Embryo. Arch. f. Anat. und Phys., Anat. Abth. II. Heft. 1887.
- 2 ZIMMERMANN, W. 1889. Über einen zwischen Aorten und Pulmonalbogen gelegenen Kiemenarterienbogen beim Kaninchen. Anat. Anz., Bd. 4.
- 3 WARREN, E. 1900. A further note on a variation in *Rana temporaria*. Anat. Anz., Bd. 18, S. 122-123.
- 4 NARATH, A. 1901. Der Bronchialbaum der Säugethiere und des Menschen. Bibliotheca medica, Abth. A, Heft 3.
- 5 MILLER, W. S. 1905. (a) The vascular system of *Necturus maculatus*. Bull. Univ. Wisconsin, no. 33, pp. 213-214.
(b) The blood and lymph vessels of the lung of *Necturus maculatus*. Am. Jour. Anat., vol. 4.
- 6 LEHMANN, H. 1905. On the embryonic history of the aortic arches in mammals. Anat. Anz., Bd. 26.
- 7 LEWIS, F. T. 1904. (a) The intra-embryonic blood-vessels of rabbits from eight and one-half to thirteen days. Am. Jour. Anat., vol. 3.
(b) The fifth and sixth aortic arches and the related pharyngeal pouches in the rabbit and pig. Anat. Anz., Bd. 28.
- 8 BREMER, JOHN L. 1902-1912. On the origin of the pulmonary arteries in mammals. (a) Am. Jour. Anat., vol. 1, no. 2, 1902.
(b) Anat. Rec., vol. 3, no. 6, 1909.
(c) The development of the aorta and aortic arches in rabbits. Am. Jour. Anat., vol. 13, p. III, 1912.
(d) An acknowledgement of Fedorow's work on pulmonary arteries. Anat. Rec., vol. 6, p. 491, 1912.

- 9 LOCY, W. A. 1906. (a) The fifth and sixth aortic arches in chick embryos, with comments on the conditions in other vertebrates. *Anat. Anz.* Bd. 29.
(b) The fifth and sixth aortic arches in birds and mammals. Cambridge, Mass. 1909.
- 10 SOULIE, A., AND BONNE, C. 1908. L' Appareil Branchiale et les Arcs Aortiques, de l'Embr. de Taupe. *Jour. de l'Anat. et de la Phys.*, no. 1.
- 11 MUDGE, G. P. 1908. An interesting case of connection between the lungs and systemic circulating system and of an abnormal hepatic blood-supply in a frog. *Jour. Anat. and Phys.*, vol. 33, pp. 54-63.
- 12 TANDLER, JULIUS 1909. Über die Entwicklung des V. Aortenbogens und der V. Schlundtasche beim Menschen. *Anat. Hefte*, no. 115.
- 13 COULTER, C. B. 1909. The early development of the aortic arches of the cat, with special reference to the presence of the fifth arch. *Anat. Rec.*, vol. 3, no. 11.
- 14 WILLIAMS, S. R. 1909. Anomalies of the pulmonary artery in *Necturus*. *Anat. Rec.*, vol. 3, no. 7, pp. 409-414.
- 15 REINKE, E. E. 1910. Note on the presence of the fifth aortic arch in a 6 mm. pig embryo. *Anat. Rec.*, vol. 4, no. 12.
- 16 REAGAN, FRANK 1912. The fifth aortic arch of mammalian embryos; the nature of the last pharyngeal evagination. *Am. Jour. Anat.*, vol. 12, no. 4.
- 17 FÖRSTER, AUGUST 1865. Die Missbildungen des Menschen systematisch dargestellt. 2. Aufl. Jena. S. 145.
- 18 SCHWALBE, ERNST Die Morphologie der Missbildungen des Menschen und der Tiere. III. Teil, III. Lief., 2. Abt., 4. Kapit. Herzheimer, Gotthold. Missbildungen des Herzens und der grossen Gefässe. Jena, 1910.
- 19 KRAUSE, W. in Henle's Gefässlehre, 1868. Varietäten der A. pulmonalis, S. 76. Varietäten des Arcus Aortae., II, 9, S. 224.
- 20 PEACOCK, 1866. Malformations. *British and Foreign med.-chir. Rev.*, vol. XXV. 1860.
Tr. Path. Soc., vol. XXVII. 1876.

PLATES

EXPLANATION OF PLATES

I to V, branchial pouches.

1 to 6, aortic arches.

7 to 14, branches of thoracic aorta entering postbranchial pulmonary plexus.

L, pulmonary anlage.

Arteries—red. Thyroid—green.

PLATE 1

Series 889, C.U.E.C. 6-mm. cat, $\times 150$. (From a reconstruction by Virginia Kneeland.)

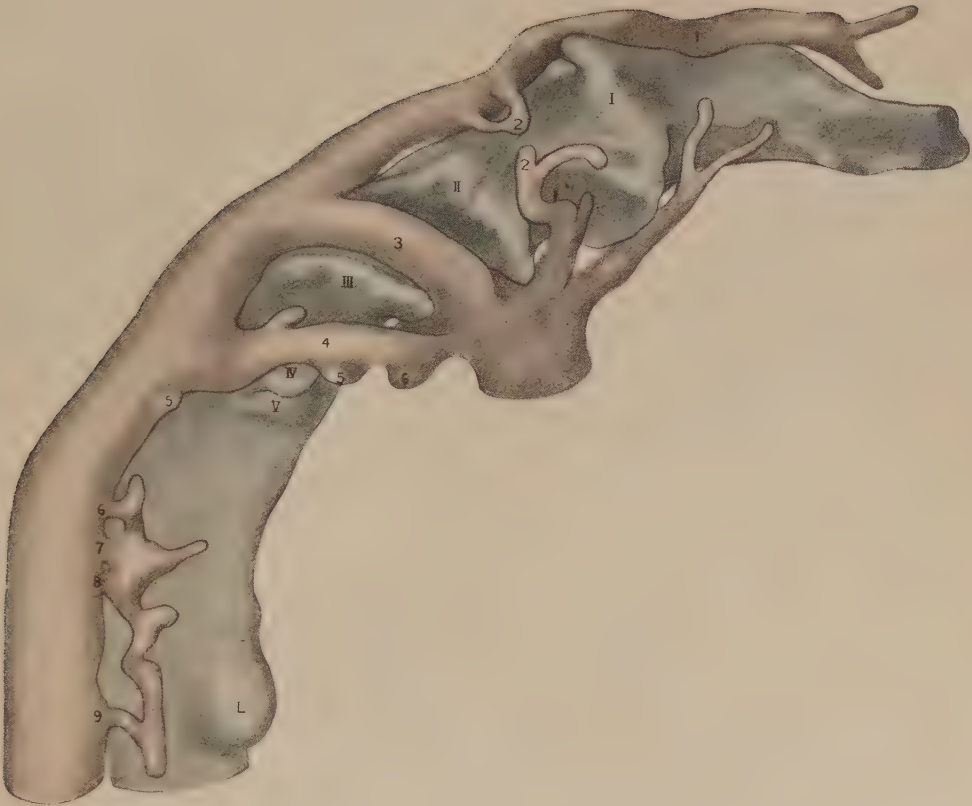


PLATE 2

Series 885, C.U.E.C. 6-mm. cat, $\times$ 150

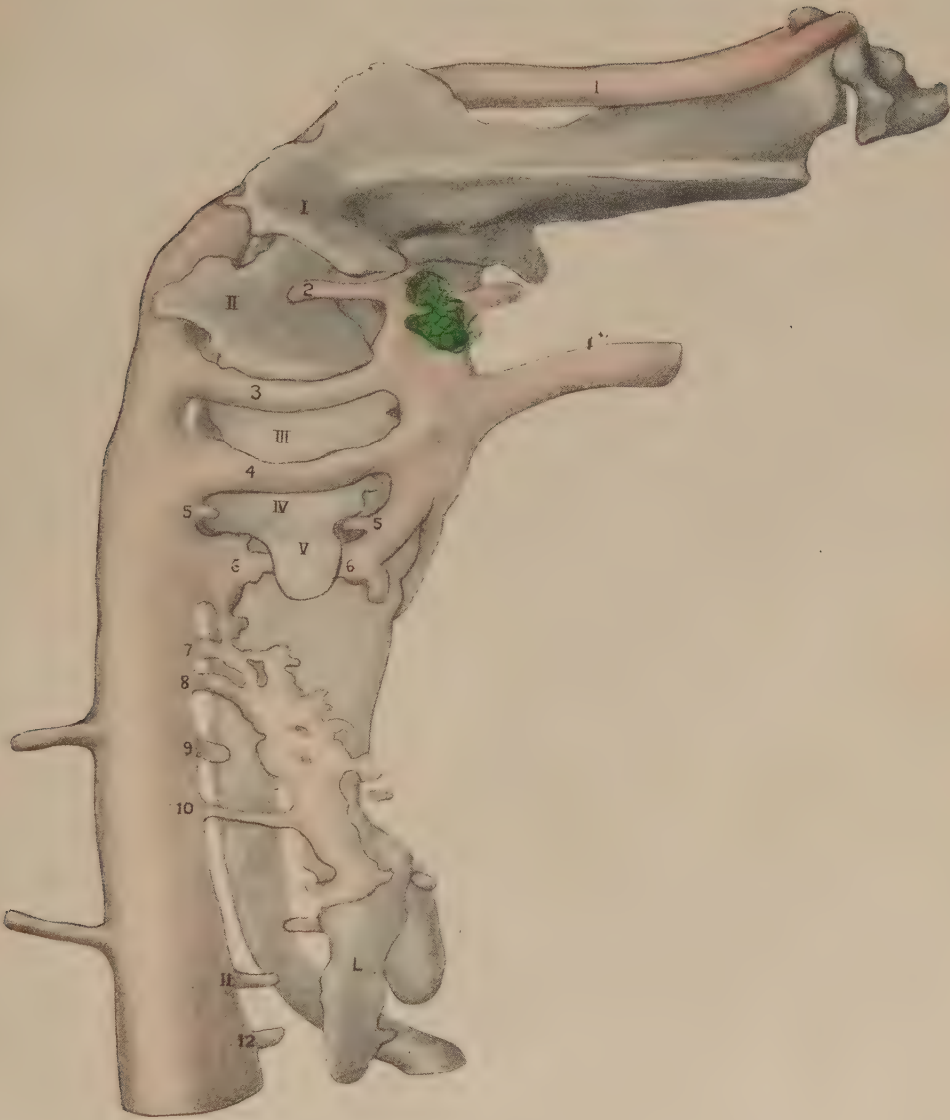


PLATE 3

Series 771, C.U.E.C. 4-mm. cat, $\times 150$

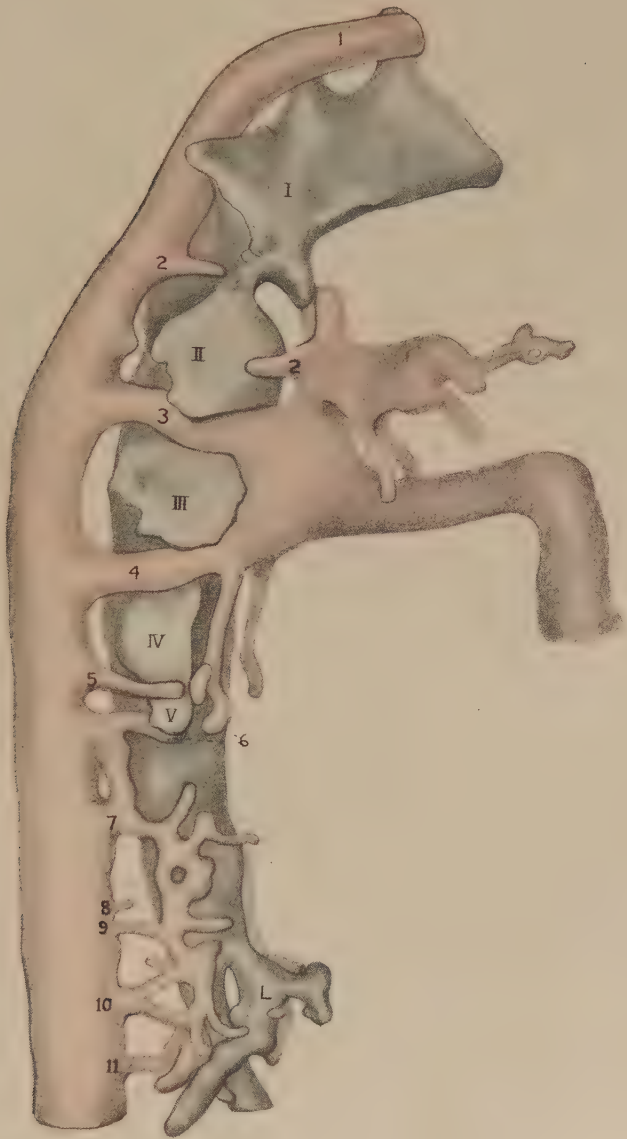


PLATE 4

Series 773, C.U.E.C. 4-mm. cat, $\times$ 150. Right side of model

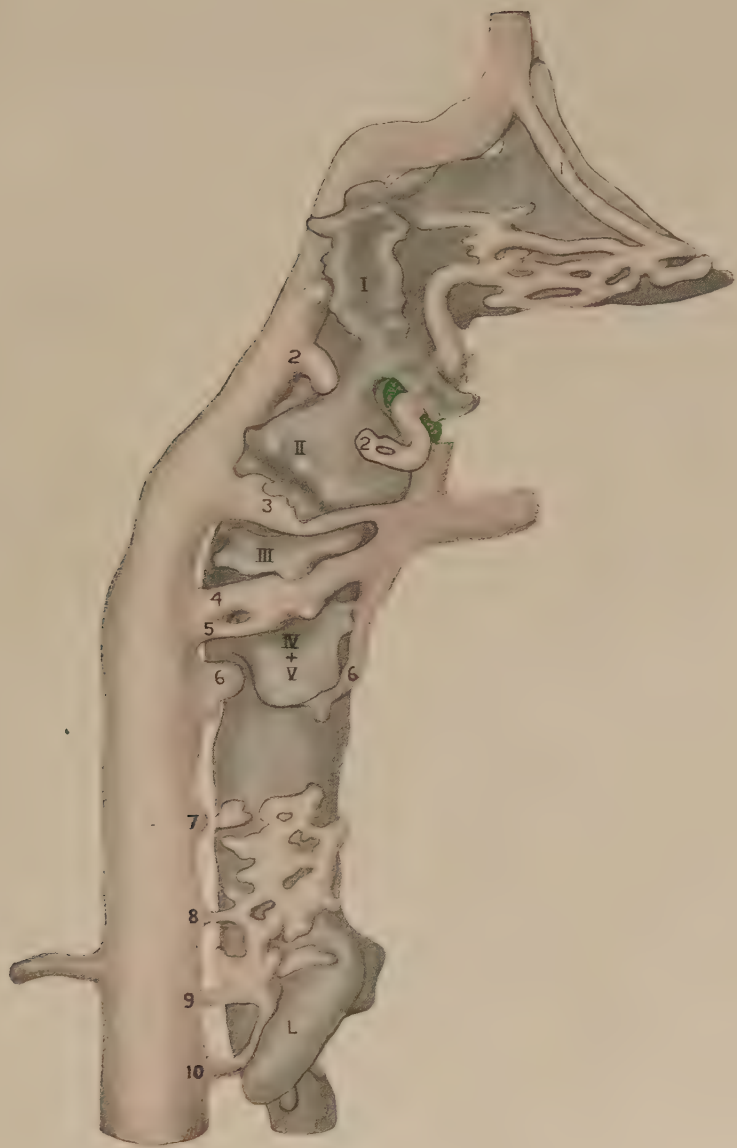
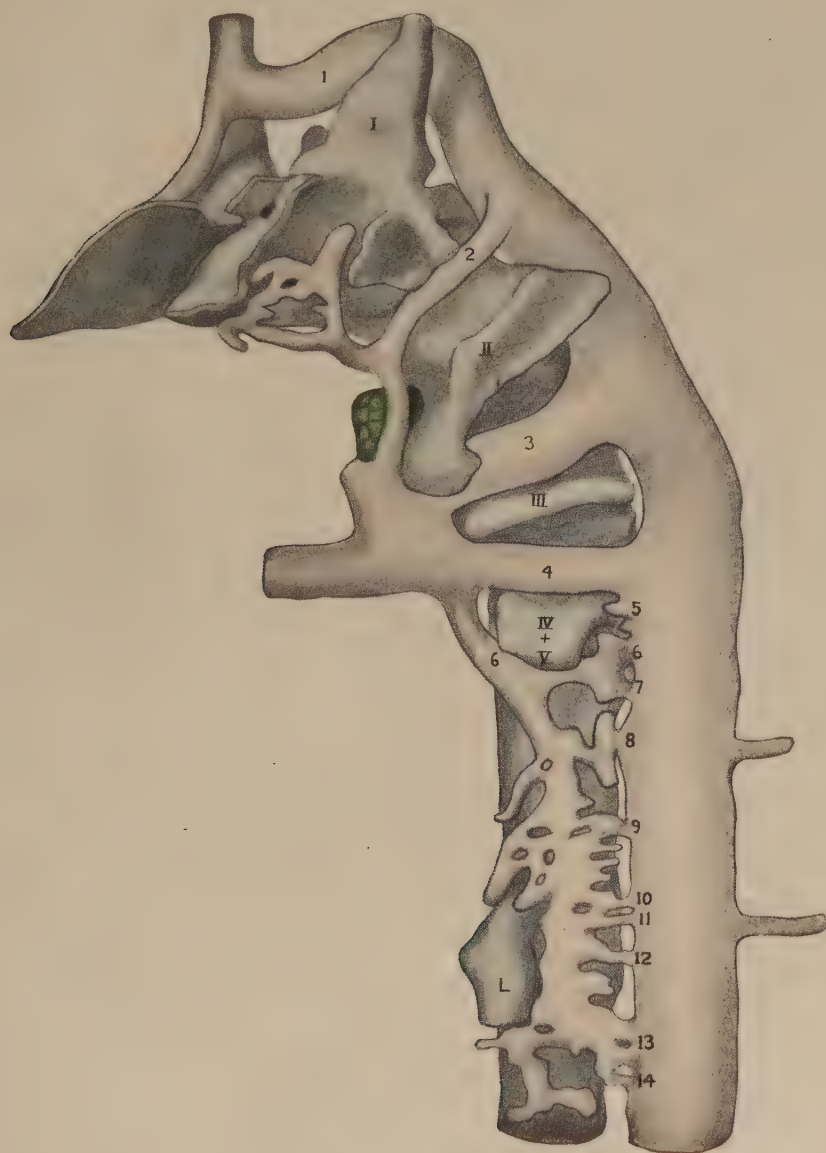


PLATE 5

Series 773, C.U.E.C. 4-mm. cat, $\times 150$. Left side of model



Resumen por el autor, Albert Kuntz,
Escuela de Medicina de San Luis.

La inervación de las gonadas del perro.

Los nervios del simpático que van a las glándulas sexuales pasan distalmente a lo largo de las arterias espermática y ovárica, respectivamente, y entran en dichas glándulas en contacto con los vasos sanguíneos o los conductos eferentes. La mayor parte de estas fibras se derivan directamente del ramo ascendente de los ganglios mesentéricos inferiores que va al plexo renal. Los vasos sanguíneos y todas las demás estructuras de las glándulas sexuales que contienen músculos lisos reciben una abundante inervación del simpático. Las pruebas obtenidas en este estudio no indican la existencia de una inervación procedente del simpático en los folículos ováricos y el tejido secretor intersticial del ovario o en el epitelio seminal y el tejido secretor intersticial del testículo. A la resección de los nervios del simpático que van al testículo sigue una degeneración del epitelio seminal y la hipertrofia simultánea del tejido secretor intersticial.

Translation by José F. Nonidez
Carnegie Institution of Washington

THE INNERVATION OF THE GONADS IN THE DOG

ALBERT KUNTZ

St. Louis University School of Medicine

FOUR FIGURES

CONTENTS

Introduction..... 203
Review of literature..... 204
 Ovary..... 204
 Testis..... 205
Material and methods..... 207
Anatomical relationships of nerves supplying the gonads..... 208
Distribution of sympathetic fibers in the ovary..... 210
 Microscopic findings..... 210
 Discussion..... 212
Distribution of sympathetic fibers in the spermatic cord and testis..... 214
 Microscopic findings..... 214
 Discussion..... 217
Summary..... 218

INTRODUCTION

The innervation of the sex glands is derived from the lumbar spinal nerves through the portions of the sympathetic trunks, including the third, fourth, fifth, and sixth lumbar ganglia. The nerves pass distally along the ovarian and spermatic arteries, respectively, and enter the glands in more or less intimate association with the blood-vessels and (in the male) the efferent ducts. The general anatomical relationships of this nerve supply are well known; however, our knowledge regarding the exact distribution of sympathetic fibers in the sex glands and the physiological significance of these fibers is still incomplete.

REVIEW OF LITERATURE

Ovary

The earlier investigators, among whom may be mentioned Frankenhäuser ('67) and Waldeyer ('70), described sympathetic nerves entering the ovary at the hilum and either accompanying the blood-vessels or passing directly to the follicles. Ellischer ('76) pointed out, further, that some of the fibers which pass to the follicles penetrate the membrana propria and terminate among the cells in the stratum granulosum. Vedeler ('90) described an abundant sympathetic nerve supply to the blood-vessels in the human ovary, but observed no fibers which enter or terminate in the follicles. Riese ('91), who studied the sympathetic nerve supply in the human ovary as well as in the ovaries of the sheep, the rabbit, the cat, and the dog, found essentially the same distribution of fibers in all cases. The nerves entering the ovary at the hilum soon became separated into two groups; the fibers of one group accompany the blood-vessels, while those of the other pass directly to the follicles. The fibers of this latter group become concentrically arranged round the follicles. Some of them penetrate the membrana propria and terminate in the stratum granulosum. Riese also described elements within the ovary which closely resemble ganglion cells, but expressed no definite conviction regarding the significance of these elements. The findings of von Herff ('92), in the human ovary, agree in general with those of Riese regarding the distribution of sympathetic fibers. Von Herff also described cellular elements within the ovary which he regarded as nervous in origin, but which do not correspond to ganglion cells in other parts of the body. Retzius ('93), deVos ('94), and Mandl ('95) described a sympathetic nerve supply to the blood-vessels in the ovary. They also observed sympathetic fibers in proximity with ovarian follicles, but none of them seemed to be of the opinion that these fibers penetrate the follicles or terminate in relation to them. Von Gawronsky ('94), who studied the nerve supply in ovaries of various mammals, described fibers which accompany the blood-vessels and fibers which pass to the follicles and, in some instances,

actually enter the stratum granulosum. He also described polygonal cells which he regarded as ganglion cells of a special type lying in the course of some of the fiber-bundles. Winterhalter ('96) described an abundant supply of sympathetic fibers, in the human ovary, both to the blood-vessels and the ovarian follicles. She emphasized the concentric arrangement of nerve-fibers within the stratum granulosum. Furthermore, she described a sympathetic ganglion within the ovary. Both von Herff and deVos ('94) had previously denied the occurrence of ganglion cells in the ovary. In 1896 von Herff again undertook an investigation of the human ovary, in order to determine the occurrence or non-occurrence in it of ganglion cells. His findings did not substantiate those of Winterhalter and he remained unconvinced of the occurrence of ganglion cells in the ovary. Markowitin ('99) also failed to find ganglion cells in the ovary. Regarding the distribution of sympathetic fibers in the ovary, his findings agree with those of the previous investigators, who described fibers actually entering the follicles. Bocura ('97) observed isolated ganglion cells in both ovaries of a woman fifty-five years of age, but could determine no essential relationship between these cells and the ovarian nerves. Furthermore, he described chromaffin cells in the stroma of these ovaries. Von Winiwarter and Sainmont ('09) and von Winiwarter ('10) observed no sympathetic fibers which penetrate the membrana propria of the ovarian follicles. They described groups of small racquet-shaped cells in the ovarian tissue which they regarded as cells which are derived from the sympathetic nervous system and are somewhat analogous with the chromaffin cells in the adrenal glands. Abel and McIlroy ('12), who studied the sympathetic nerve supply in the ovaries of the dog, the cat, and the rabbit, observed no cells which could be regarded as ganglion cells. According to their observations, the nerves, upon entering the ovary at the hilum, become separated into three sets, viz., a vascular, a follicular, and an interstitial set, all of which anastomose with each other. The follicular nerves lie in the tunicae externa and interna and do not penetrate the stratum granulosum. Brill ('15) described and illustrated a well-developed sympathetic

ganglion in the ovaries of the mouse and the rabbit which lies in the stroma and is intimately associated with the fiber-bundles which enter the ovary at the hilum. Within this ganglion he described numerous elements which he regarded as chromaffin cells. Furthermore, he described fibers included in the fibrous network within the ganglion which apparently terminate on these chromaffin cells. Brill's description of the supply of sympathetic fibers to the blood-vessels in the ovary is in essential agreement with the findings of other investigators. He also described an abundant supply of fibers to the interstitial secretory tissue and even traced sympathetic fibers into the corpus luteum where they terminate in luteim cells. In the ovarian follicles he observed sympathetic fibers not only in the theca folliculi, but also in the stratum granulosum. Some of these fibers, he said, penetrate so deeply into this stratum that they actually touch the cells in the innermost layer. According to Ramon ('18), the nervous elements in the ovary form a ganglionated plexus.

Testis

As early as 1868 Letzerich recorded observations according to which he traced fibers from nerves lying in the connective tissue between the seminiferous tubules, through the membrana propria, to their terminations on cells in the deeper layers of the seminal epithelium. These fibers, he said, terminate in pyramidal masses of protoplasm which contain glistening granules and elliptical nuclei and are enveloped by a membrane which is probably a continuation of the nerve sheath. Retzius ('93), employing the Golgi method on the testes of the cat, was unable to substantiate the findings of Letzerich. He observed nerve-fibers which, he believed, penetrate the membrana propria in small numbers, but was unable to observe the terminations of these fibers among the cells of the seminal epithelium. Timofeew ('94), who studied the distribution of the sympathetic fibers in the spermatic cord and testis of the rabbit, the guinea-pig, the rat, the cat, and the dog, described a rich plexiform network of fibers associated with the blood-vessels, the ductus deferens, and the ductus epididymis.

He also observed nerve fibers closely associated with the seminiferous tubules, but was unable to find any fibers which penetrate the *membrana propria*. He regarded the fibers which appear on the surface of the seminiferous tubules as fibers which supply small blood-vessels. Cavalie ('02) described a rich plexiform network of sympathetic fibers associated with the blood-vessels and the seminiferous tubules both in the testis of the fowl and the rabbit. He also described arborizations formed by these fibers about the cells in the deepest layers of the seminal epithelium and, in the rabbit, about the epithelial cells in the walls of the ductus epididymis. Loisel ('02) described fiber terminations in the deep layers of the walls of the seminiferous tubules both on spermatogenic and sustentacular cells.

MATERIAL AND METHODS

Careful dissections were made in a number of dogs to determine as accurately as possible the anatomical relationships of the nerves which supply the sex glands. In order to determine more accurately the sources of the sympathetic fibers to the testis and to observe the results of resection of these fibers, operations in which the inferior mesenteric ganglia were removed and the nerves descending along the right spermatic artery were resected as completely as possible without injury to the walls of the vessel were performed aseptically on dogs.¹ These animals were killed after intervals of from twenty-one to thirty days. The spermatic cords and testes were removed. Parts of the tissue were prepared by the pyridine-silver method and parts by ordinary staining processes.

For the study of the distribution of the sympathetic fibers in the gonads, both ovaries and testes of unoperated dogs were prepared by the pyridine-silver method.

¹ The writer desires to acknowledge his indebtedness to Dr. J. E. Thomas for performing these operations.

ANATOMICAL RELATIONSHIPS OF THE NERVES SUPPLYING THE GONADS

By the aid of physiological and histological methods, including a study of the distribution of degenerated myelinated fibers following section of the nerves, Langley and Anderson ('95 and '96) have shown, in the cat and the rabbit, that the nerve supply to the internal generative organs is derived from the second, third, fourth, fifth, and sixth lumbar nerves. By careful dissection they traced rami from the fourth, fifth, and sixth lumbar ganglia of the sympathetic trunks into the inferior mesenteric ganglia, and from the third lumbar ganglia of the sympathetic trunks into the superior mesenteric ganglia. From the latter ganglia fibers pass into the renal plexuses. They also described a small ganglion, present in the majority of cases, on the ramus from the fourth lumbar sympathetic ganglion at or near the point of junction of this ramus with the ramus from the fifth lumbar sympathetic ganglion. This ganglion, which they have designated the ovarian (spermatic) ganglion, lies in proximity with the ovarian (spermatic) artery. It receives filaments from the ramus ascending from the inferior mesenteric ganglion to the renal plexus and sends filaments distally along the ovarian or spermatic artery.

My observations on the anatomical relationships of the nerves supplying the gonads in the dog agree in all essential respects with the findings of Langley and Anderson in the cat and the rabbit. Figure 1 is based on a number of dissections and is an attempt to illustrate the relationships most commonly found in dogs. The third lumbar ganglion of the sympathetic trunk sends one or more rami into the superior mesenteric ganglion. This ganglion sends slender rami into the renal plexus and is connected by a larger ramus with the inferior mesenteric ganglia. The fourth, fifth, and sixth lumbar ganglia of the sympathetic trunk commonly send rami into the inferior mesenteric ganglia. The ramus from the sixth lumbar ganglion is sometimes absent. In such cases a ramus arises from the sympathetic trunk between the fifth and sixth lumbar ganglia. In some cases a ramus was also found to arise from the sympathetic trunk just above the

fourth lumbar ganglion. The latter ramus commonly joins the ramus ascending from the inferior mesenteric ganglia to the renal plexus. In those cases in which an ovarian (spermatic) ganglion is present it is commonly located at this point of junction which is in proximity with the ovarian (spermatic) artery. From this ganglion and from the ascending ramus from the inferior mesenteric ganglia slender rami may be traced to the ovarian or spermatic artery. These observations indicate that the majority of the fibers passing distally along the ovarian or spermatic arteries come from the inferior mesenteric ganglia via the ascending

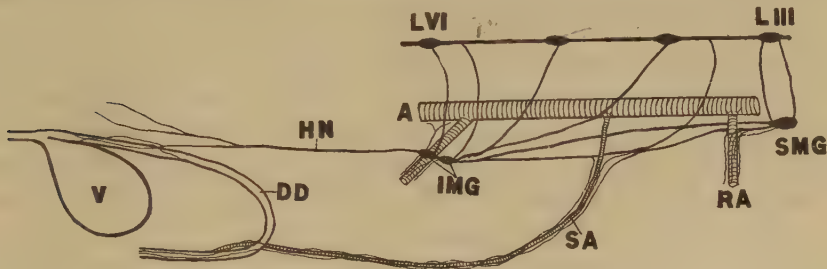


Fig. 1. Diagram illustrating the anatomical relationships of the sympathetic nerves to the sex glands. A., aorta; D.D., ductus deferens; H.N., hypogastric nerve; I.M.G., inferior mesenteric ganglia; LIII to VI, lumbar ganglia of sympathetic trunk; R.A., renal artery; S.A., spermatic artery; S.M.G., superior mesenteric ganglion; V., vesicle.

ramus. This conclusion is substantiated also by a study of degenerative changes in the testes following the removal of the inferior mesenteric ganglia without resection of the nerves descending along the spermatic artery. Degenerative changes in the testis following this operation were practically identical with those following the removal of the inferior mesenteric ganglia and resection of the nerves descending along the spermatic artery. The hypogastric nerve supplies some fibers to the pelvic end of the ductus deferens, but these fibers probably do not reach the testis.

DISTRIBUTION OF SYMPATHETIC FIBERS IN THE OVARY

Microscopic findings

Ovaries of the dog prepared by the pyridine-silver method show a relatively abundant supply of sympathetic nerves. Upon entering at the hilum these nerves break up into branches which radiate toward all parts of the cortex either intimately associated with or in proximity with blood-vessels. The ovary is a highly vascular organ. The arteries which radiate from the hilum to all parts of the cortex are characterized by their tortuous course in the medulla and their thick muscular walls. The veins are numerous and relatively large. In sections of the ovary the majority of the sympathetic fiber-bundles occur in proximity with blood-vessels. These bundles supply fibers to the smooth muscle which is so abundant in the walls of the arteries. Small bundles and isolated fibers occur frequently in the fibromuscular tissue of the stroma without any apparent relationship to blood-vessels. Many of these fibers terminate in the fibromuscular tissue on smooth muscle cells. Figure 2, A, is a camera-lucida sketch illustrating sympathetic fibers associated with a blood-vessel in the stroma. The stippled portion of the vessel lies just beneath the surface in the section. Figure 2, B, is a camera-lucida sketch illustrating sympathetic fibers which ramify in the fibromuscular tissue of the stroma without apparent relationship to blood-vessels. Near the periphery of the medulla and in the deeper layers of the cortex many sympathetic fibers come into very close proximity with aggregates of interstitial secretory elements, but careful observation failed to reveal any fibers which terminate on interstitial secretory cells. As the fiber-bundles, becoming more slender toward the periphery of the ovary, are traced into the cortex, many of them come into proximity with the ovarian follicles, some even deviating from their radial course round the larger follicles. No sympathetic fibers were observed in the peripheral layers of the cortex except in close proximity with blood-vessels. A painstaking, systematic search failed to reveal any nerve-fibers which penetrate the membrana propria of the ovarian follicles. In some instances sympathetic fibers

appear to be in the theca folliculi. Doubtless, these are to be regarded as fibers which pass over or under the follicles in their course along peripheral blood-vessels. In areas of the cortex in which the interstitial secretory tissue is best developed sympathetic fibers are relatively rare. They do not penetrate these areas except as they accompany the small arteries which pass through or supply the interstitial tissue. In ovaries which



Fig. 2. (A) Camera-lucida sketch of nerve-fibers associated with a blood-vessel in the stroma ovarii. The stippled portion of the vessel lies just beneath the surface in the section. (B) Camera-lucida sketch of nerve-fibers ramifying in the stroma ovarii without apparent relationship with blood-vessels.

contain well-developed corpora lutea a few sympathetic fibers may, in some instances, be traced along the larger blood-vessels which lie in the connective tissue between the columns of lutein cells. None of these fibers were observed to deviate from the connective-tissue septa and assume a relationship with the lutein cells. Neither could nerve fibers be observed in areas of the connective-tissue septa which contained no blood-vessels except capillaries.

Although a systematic search for ganglion cells was made in a number of ovaries prepared by the pyridine-silver method, none were observed. In sympathetic ganglia prepared in precisely the same manner, the ganglion cells are well impregnated. Therefore, the conclusion seems to be warranted that ganglion cells do not commonly occur in the ovary of the dog.

Discussion

A review of the literature bearing on the innervation of the mammalian ovary reveals the fact that the majority of the investigators in this field have described a sympathetic nerve supply to the ovarian follicles as well as to the blood-vessels in the ovary. Among the more recent investigators, Abel and McIlroy ('12) and Brill ('15) also described a sympathetic nerve supply to the interstitial secretory tissue. Brill further described sympathetic fibers which terminate on secretory elements of the corpus luteum.

A priori, there is no apparent demand for a sympathetic nerve supply to any tissue in the ovary except the smooth muscle which occurs in the walls of the blood-vessels and in the fibromuscular tissue of the stroma. Furthermore, the results of ovarian transplantation and other experimental work speak against the occurrence of a sympathetic nerve supply to the ovarian follicles and the interstitial secretory tissue. Therefore, the acceptance of a sympathetic nerve supply to any tissue in the ovary, except the smooth muscle, as a demonstrated fact is not justifiable unless the evidence satisfies the most rigid requirements.

The writer's observations recorded above indicate an abundant supply of sympathetic fibers to the smooth muscle in the walls of the ovarian blood-vessels and the fibromuscular tissue of the stroma ovarii, but afford no evidence which indicates a sympathetic nerve supply either to the ovarian follicles or to the interstitial secretory tissue. Negative evidence is not conclusive. However, these observations were made on pyridine-silver preparations in which the nerve-fibers accompanying the blood-vessels

and ramifying throughout the stroma ovarii are impregnated so successfully that they can be traced without difficulty. If fibers of the same character were present in and around the ovarian follicles and in the areas of interstitial secretory tissue, they could hardly have escaped observation.

Mere proximity of nerve-fibers with aggregates of cells of a given type does not prove a physiological relationship between these two types of tissue elements. Furthermore, any one who has employed silver-impregnation methods extensively knows that silver is precipitated also in tissues which are not nervous in character and that such tissues thus impregnated may resemble nervous tissue very closely. The observer must, therefore, differentiate between nervous and non-nervous elements. The writer has frequently observed concentric lines round ovarian follicles which somewhat resemble non-medullated nerve fibers, but lack the clear-cut appearance and the tortuous course which are so characteristic of well-impregnated sympathetic fibers. An exhaustive study convinces me that these structures are not nervous in character. I am also convinced, by a study of the literature reviewed in an earlier section of this paper, that these or similar structures have frequently been described as sympathetic nerve-fibers which innervate the ovarian follicles.

Ganglion cells or a circumscribed ganglion within the ovary have been described by but few investigators, while others recorded the fact that they were unable to find such elements. Obviously, ganglion cells do not occur uniformly in mammalian ovaries. Ganglion cells in the ovary, wherever this phenomenon occurs, doubtless, are to be regarded as elements which, during embryonic life, became displaced from aggregates of sympathetic neurones which are more centrally located, but send their axones into the ovary as components of the ovarian nerves.

DISTRIBUTION OF SYMPATHETIC FIBERS IN THE SPERMATIC CORD
AND TESTIS*Microscopic findings*

In a cross-section of the spermatic cord as it traverses the inguinal canal not less than thirty bundles of nerve-fibers, ranging from slender filaments to bundles containing upward of one hundred non-medullated fibers, occur more or less intimately associated with the ductus deferens. No fiber-bundles of considerable size occur within the muscular layers of the wall of the ductus deferens, but terminations on muscle-fibers are numerous. Throughout the remaining areas of the section bundles of non-medullated nerve-fibers occur in considerable numbers, especially in proximity with blood-vessels. These bundles show approximately the same range of variation in the number of fibers as do those associated with the ductus deferens. The distribution of fiber-bundles in a typical cross-section of the spermatic cord as it traverses the inguinal canal is illustrated in figure 3. The distribution of these fibers as observed in transverse sections taken at various levels of the spermatic cord and also in longitudinal sections of segments of the cord suggest a relatively intricate plexiform network and an abundant nerve supply.

In transverse sections taken farther distally relatively small bundles of fibers occur associated with the ductus epididymis. These bundles supply fibers to the thin layer of smooth muscle in the wall of this duct. Among the ductuli efferentes the majority of the slender sympathetic filaments present are closely associated with blood-vessels, but also supply fibers to the very thin layer of smooth muscle in the walls of these ductules.

In the mediastinum testis as well as in the connective tissue between the seminiferous tubules slender bundles of sympathetic fibers occur in proximity with blood-vessels. Nerve-fibers which penetrate the membrana propria of the seminiferous tubules to terminate either on spermatogenic or sustentacular cells were not observed. In like manner, an exhaustive search failed to reveal any sympathetic fibers which terminate on interstitial secretory cells. The general distribution of sympathetic fibers

in the testis is apparently determined by the distribution of arteries and veins. The relationship of fiber-bundles to blood-vessels and ductuli efferentes in a limited area of a section is illustrated in figure 4. Areas of connective tissue between the seminiferous tubules which contain no blood-vessels except capillaries usually contain no sympathetic fibers. Neither do sympa-



Fig. 3. Diagram made with the aid of the camera-lucida to illustrate the distribution of sympathetic fiber-bundles in a cross-section of the spermatic cord. The solid black areas indicate sympathetic fiber-bundles. The arteries are indicated with heavy and the veins with light walls. D.D., ductus deferens.

thetic fibers invade areas of interstitial secretory tissue except as they accompany blood-vessels. If sympathetic fibers were supplied to the seminal epithelium or the interstitial secretory elements, the distribution of such fibers in the connective tissue between the seminiferous tubules would, doubtless, be more uniform. There would probably be no areas of considerable size in which sympathetic fibers could not be found.

Numerous sympathetic rami of considerable size occur in the tunicae albuginea and vasculosa. These rami run a somewhat tortuous course, and in transverse sections of the testis do not always appear in close proximity with blood-vessels; however, fiber terminations other than those occurring in the muscular layers of the walls of the blood-vessels were not observed.

As indicated above, dogs were subjected to an operation in which the inferior mesenteric ganglia were removed and the nerves descending along the right spermatic artery were resected.

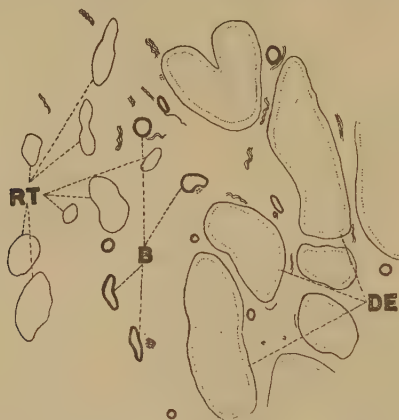


Fig. 4. Outline drawing made with the aid of the camera-lucida to illustrate the distribution of sympathetic fibers with reference to blood-vessels, ductuli efferentes, and rete testis in a small area of a cross-section of the testis. *B.*, blood-vessels; *D.E.*, ductuli efferentes; *R.T.*, rete testis.

These dogs were killed after intervals of from twenty-one to thirty days following operation. No gross changes were observed at autopsy except marked congestion of the blood-vessels in the spermatic cords and a somewhat hyperaemic condition of the testes. In pyridine-silver preparations of the right spermatic cord and testis the great majority of the sympathetic fibers show evidence of degeneration. In sections of the testes prepared by the ordinary staining processes the seminiferous tubules show advanced degenerative changes and an accompanying hypertrophy of the interstitial secretory tissue. These changes will be described in another paper.

Discussion

Among the investigators whose work on the distribution of sympathetic nerve-fibers in the testis is reviewed in an earlier section of this paper, Timofeew alone failed to describe structures which might be interpreted as sympathetic fibers which penetrate the membrana propria of the seminiferous tubules and terminate among the cells of the seminal epithelium. All the other investigators described a sympathetic nerve supply both to the smooth muscle in the walls of blood-vessels and ducts and to the epithelium of the seminiferous tubules.

The writer's observations recorded above indicate an abundant supply of sympathetic fibers to all the structures in the spermatic cord and testis which contain smooth muscle, but afford no evidence which indicates a sympathetic nerve supply either to the epithelium of the seminiferous tubules or to the interstitial secretory tissue. Negative evidence is necessarily inconclusive; however, in the material studied, the sympathetic fibers in the spermatic cord and testis described above were impregnated so successfully that if a sympathetic nerve supply to the epithelium of the seminiferous tubules or the interstitial secretory tissue were present, it could hardly have escaped observation.

Degeneration of the seminal epithelium following the elimination of the sympathetic nerve supply to the testis is very similar (probably identical) in type to the degeneration of this tissue described by Barratt and Arnold ('11) following exposure of the testes to x-rays and to that described by Allen ('19) in albino rats which had been subjected to a diet deficient in the water-soluble vitamine. It does not indicate that the normal functioning of this tissue requires a sympathetic nerve supply. Degeneration of the seminal epithelium in these dogs is probably the result of metabolic disturbances due to paralysis of the blood-vessels in the spermatic cord and testis.

Hypertrophy of the interstitial secretory tissue in the testes of these dogs probably has no direct relationship to the elimination of the sympathetic nerve supply to the testis, but is an accompaniment of the degenerative changes in the seminal

epithelium. Hypertrophy of the interstitial secretory tissue is known to be a common accompaniment of degeneration of the seminal epithelium following ligature or occlusion of the ductus deferens, prolonged exposure of the testes to the x-rays, etc. It is probably an accompaniment of degeneration of the seminal epithelium due to any cause which does not result in injury to or impoverishment of the interstitial tissue. Obviously, the growth and functioning of this tissue do not require sympathetic innervation. This conclusion is also in accord with the results of testicular transplantation and other experimental work.

SUMMARY

The sympathetic nerves to the sex glands pass distally along the ovarian and spermatic arteries, respectively, and enter the glands in more or less intimate association with the blood-vessels or the efferent ducts. The majority of these fibers are derived directly from the sympathetic ramus ascending from the inferior mesenteric ganglia to the renal plexus.

The blood-vessels and all other structures in the sex glands which contain smooth muscle receive an abundant sympathetic nerve supply. The evidence available does not indicate a sympathetic nerve supply either to the ovarian follicles and the interstitial secretory tissue in the ovary or to the seminal epithelium and the interstitial secretory tissue in the testis.

Resection of the sympathetic nerves to the testis is followed by degeneration of the seminal epithelium and hypertrophy of the interstitial secretory tissue. The degenerative changes are probably due to paralysis of the blood-vessels in the spermatic cord and testis. Hypertrophy of the interstitial secretory tissue is an accompaniment of degeneration of the seminal epithelium.

LITERATURE CITED

- ABEL W., AND McILROY, A. L. 1912 The arrangement and distribution of nerves in certain mammalian ovaries. *Proc. Roy. Soc.*, 1912-13, vol. 6, Obst. and Gyn. Sec., pp. 240-247.
- ALLEN, E. 1919 Degeneration in the albino rat testis due to a diet deficient in the water-soluble vitamine, with a comparison of similar degeneration in rats differently treated, and a consideration of the Sertoli tissue. *Anat. Rec.*, vol. 16, pp. 93-117.

- BOCURA, K. 1907 Nachweis von chromaffinem Gewebe und wirklichen Ganglienzellen im Ovar. Wien. klin. Woch., No. 28, 1907.
- BARRATT, W., AND ARNOLD, G. 1911 Cell changes in the testis due to x-rays. Archiv f. Zellforschung, Bd. 7.
- BIEDL, A. 1913 Internal secretory organs. New York, 1913.
- BRILL, W. 1915 Untersuchungen über die Nerven des Ovariums. Arch. f. mikr. Anat., Bd. 86, sec. 1, S. 338-344.
- CAVALIE 1902 Terminaisons nerveuses dans le testicule chez le lapin et chez le poulet, et dans l'epididyme chez le lapin. Comp. Rend. Soc. de Biol., Par., T. 11, s. IV, pp. 289-300.
- DE VOS, J. 1894 Etude sur l'innervation de l'ovaire. Bull. Acad. de Med. Belg., Ser. IV, T. 8.
- FRANKENHÄUSER 1867 Die Nerven der Gebärmutter und ihre Endigungen in den glatten Muskelfasern. Jena, 1867.
- LANGLEY, J. N., AND ANDERSON, M. B. 1896 The innervation of the pelvic and adjoining viscera. Jour. Physiol., pp. 372-406.
- LOISEL, G. 1902 Terminaisons nerveuses et éléments glandulaires de l'épithélium séminifère. Comp. Rend. Soc. de Biol., T. 54, p. 346.
- MANDL 1895 Über Anordnung und Endigungsweise der Nerven in Ovarium. Arch. f. Gyn., Bd. 48, S. 376.
- RAMON 1918 Contribucion al estudio de la inervacion ovarica. Aragon medico, May, 1918. Abstract in Endocrinology, vol. 3, p. 84.
- RETZIUS 1893 Über die Nerven der Ovarien und Hoden. Biol. Untersuch., Bd. 5, S. 31.
- RIESE, H. 1891 Die feinsten Nervenfasern und ihre Endigungen im Ovarium der Säugethiere und des Menschen. Anat. Anz., Bd. 6, S. 400-420.
- TIMOFEEW, D. 1894 Zur Kenntniss der Nervenendigungen in der männlichen Geschlechtsorganen der Säuger. Anat. Anz., Bd. 9, S. 342-348.
- VEDELER 1890 Nerven imenneske ovariet. Norsk. Mag. Lægevid., vol. 51
- VON GAWRONSKY 1894 Über Verbreitung und Endigung der Nerven in den weiblichen Genitalien. Arch. f. Gyn., Bd. 47, S. 271.
- VON HERFF 1892 Über den feineren Verlauf der Nerven im Eierstocke des Menschen. Zeitschr. Geb. u. Gyn., Bd. 24, S. 289.
1896 Gibt es ein sympathisches Ganglion im menschlichen Ovarium? Arch. f. Gyn., Bd. 51, S. 374-388.
- VON WINIWARTER, H., ET SAINMONT 1909 Nouvelles recherches sur l'ovogenèse et l'organogenese de l'ovaire des mammiferes. Arch. de Biol., T. 25, p. 627.
- VON WINIWARTER, H. 1910 Contribution à l'étude de l'ovaire humain. Arch. de Biol., T. 25, pp. 683-756.
- WALDEYER 1870 Eierstock und Ei. Leipsic, 1870.
- WINTERHALTER, E. H. 1896 Ein sympathisches Ganglion im menschlichen Ovarium. Arch. f. Gyn., Bd. 51, S. 49-55.

Resumen por el autor, Albert Kuntz,
Escuela de Medicina de San Luis.

Degeneración experimental del testículo del perro.

A la eliminación de la inervación procedente del simpático, en el testículo, sigue la degeneración del epitelio seminal y la hipertrofia simultánea del tejido secretor intersticial. Esta degeneración es de carácter semejante a la degeneración del tejido seminal provocada por una exposición de los testículos a los rayos X, una dieta insuficiente en vitamina soluble en el agua, etc. La degeneración del epitelio seminal debida a la misma causa y la hipertrofia asociada del tejido secretor intersticial se iniciaron más tarde y progresaron con menor rapidez en perros en los cuales se extrajo el testículo izquierdo que en los en que se dejaron in situ ambos testículos. Por consiguiente la hipertrofia del tejido secretor intersticial se inicia probablemente más pronto como un fenómeno que acompaña a la degeneración del epitelio seminal que como un proceso compensador debido a la extracción de uno de los testículos, si es que tal hipertrofia compensadora existe realmente. La ligadura y resección del conducto deferente derecho en cuatro perros provocó la degeneración del epitelio seminal y una hipertrofia simultánea del tejido secretor intersticial de ambos testículos. La degeneración observada en el testículo izquierdo en estos casos está indudablemente asociada o condicionada por la degeneración del testículo derecho.

Translation by José F. Nonidez
Carnegie Institution of Washington

EXPERIMENTAL DEGENERATION IN THE TESTIS OF THE DOG

ALBERT KUNTZ

St. Louis University School of Medicine

FOUR FIGURES

CONTENTS

Introduction.....	221
Material and methods.....	221
Changes following elimination of the sympathetic nerve supply.....	222
Changes following ligature and resection of the right ductus deferens.....	226
Discussion.....	229
Summary.....	233

INTRODUCTION

While studying the distribution of nerve fibers in the testes of dogs in which the inferior mesenteric ganglia had been removed and the nerves descending along the right spermatic artery resected, unexpected degenerative changes were observed in the epithelium of the seminiferous tubules. In order to secure material in which degeneration of the seminal epithelium due to some other known cause might be observed, dogs were subjected to an operation in which the right ductus deferens was ligatured and resected. The degenerative changes observed in the testes of both series of dogs were so striking that it seemed desirable to describe them in this brief paper because of their bearing on the general physiology of the male generative organs.

MATERIAL AND METHODS

The material used in this study was obtained from eight dogs, all of which were subjected to operation under aseptic conditions.¹ These dogs were secured from the municipal pound. Nothing

¹ The writer desires to acknowledge his indebtedness to Dr. J. E. Thomas for performing these operations.

is known of their previous history, but all were apparently normal, vigorous animals. In the first series of four dogs, operated under general anesthesia, the inferior mesenteric ganglia were removed and the nerves descending along the right spermatic artery were resected as completely as possible without injury to the vessel. In dogs nos. 3 and 4 of this series the left testis was removed at the time of operation and prepared for study. In the second series of four dogs the right ductus deferens was ligatured and resected. In dogs nos. 5 and 6 the ductus deferens was secured through an abdominal incision. No. 5 was operated under general, and no. 6 under local anesthesia. In dogs nos. 7 and 8 the right ductus deferens was secured through an incision just proximal to the scrotum. These dogs were not anesthetized, but were operated under the influence of a moderate dose of morphine. All of the dogs recovered from the operation without incident and were killed at intervals of from twenty to thirty days after operation. Parts of both testes of each dog were prepared for study. The material used for the study of degeneration phenomena was prepared by the ordinary haematoxylin-eosin method.

CHANGES FOLLOWING ELIMINATION OF THE SYMPATHETIC NERVE SUPPLY

Dog no. 1 was subjected to operation on January 24th. The inferior mesenteric ganglia were removed and the nerves descending along the right spermatic artery were resected. The animal was killed on February 14th, i.e., twenty-one days after operation. No gross changes were observed at autopsy except that the blood-vessels in both spermatic cords were congested and the testes were somewhat hyperaemic.

In tranverse sections of the spermatic cords the blood-vessels appear fully dilated and contain masses of blood-cells. In sections of the testes the blood-vessels are also dilated and the epithelium of the seminiferous tubules shows well-marked degenerative changes. In many of the tubules epithelial cells, either isolated or in small masses, lie free in the lumen. The cells in the epithelium are materially reduced in number, and many of

those remaining show well-marked necrotic changes. Spermatogenesis has ceased and spermatids are present only in small numbers. Part of a section of one of these tubules is illustrated in figure 1. As indicated in this figure, the degenerative changes have involved all of the components of the seminal epithelium. Mature spermatozoa are absent. The few remaining spermatids are obviously necrotic. Spermatocytes are relatively few. Some of these appear quite normal, while others are obviously necrotic. The spermatogonia are also reduced in number, but those remaining show no well-marked changes. The nuclei of the sustentacular cells appear quite normal. Cytoplasmic changes which suggest reticular degeneration are apparent in all parts of the epithelium. In some of the tubules which show

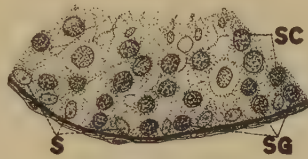


Fig. 1 Section of the wall of a seminiferous tubule in the right testis of dog no. 1. *S.*, nuclei of sustentacular cells; *SC.*, spermatocytes; *SG.*, spermatogonia.

less advanced degenerative changes extensive destruction of epithelial elements is not apparent, although free cells occur in small numbers in the lumen. Spermatozoa usually with their heads imbedded in the cytoplasm of the sustentacular cells are present in small numbers. Many of the spermatids present are obviously necrotic. The nuclei of the majority of the other elements appear quite normal, but cytoplasmic changes are well-marked throughout the epithelium. Changes in the interstitial connective tissue cannot be observed, but the interstitial secretory tissue has undergone apparent hypertrophy.

Dog no. 2 was subjected to operation on February 4th. The inferior mesenteric ganglia were removed and the nerves descending along the right spermatic artery were resected. This dog was killed on March 4th, i.e., twenty-eight days after operation. No gross changes were observed at autopsy except that

the blood-vessels in the spermatic cords were congested and the testes were somewhat hyperaemic.

Upon microscopic examination, the blood-vessels in the spermatic cords and testes appear fully dilated, both arteries and veins containing masses of blood-cells. The seminiferous tubules show advanced degenerative changes (fig. 2). In many of these tubules the epithelium is almost completely destroyed and a degeneration reticulum extends well into the lumen, in many instances occluding it. Fragmenting epithelial cells, either isolated or in small aggregates, lie in the reticulum or in the lumen. The majority of the few spermatogenic elements which remain in the epithelium are spermatogonia. Isolated primary spermatocytes are still present in nearly all of the tubules. Some of the tubules show a larger number of primary spermatocytes and a relatively small number of secondary spermatocytes. Spermatids are practically absent and spermatozoa were not observed either in the seminiferous tubules or in any of the efferent ducts. The majority of the spermatogenic elements still present are becoming necrotic, as is evidenced by the fragmentation of the chromatin material in their nuclei and its lack of affinity for basic stains. The nuclei of the sustentacular cells are very apparent because they are not obscured by spermatogenic elements. The majority of these nuclei have receded somewhat from the membrana propria toward the lumen. Many of them are somewhat irregular in outline and obviously becoming necrotic. These elements are probably reduced in number, but to a lesser extent than any of the spermatogenic elements. The cytoplasm of the sustentacular cells is fragmented and incorporated in the general degeneration reticulum. Although there may be a slight increase in the amount of interstitial connective tissue, the evidence on this point is not conclusive. The interstitial secretory tissue is markedly increased (fig. 2), and in some areas of the sections shows evidence of active hyperplasia. Nothing was observed which indicates degeneration of the interstitial tissue.

In this case as well as in the case of dog no. 1, although the nerves descending along the left spermatic artery were not

resected, the left testis showed degenerative changes in the seminal epithelium indential in character and quite as advanced as the right testis. It also shows corresponding hypertrophy of the interstitial secretory tissue.

Dogs nos. 3 and 4 were subjected to the same operation as dogs nos. 1 and 2, except that the left spermatic cord was ligatured just proximal to the testis and the left testis was removed. Dog no. 3 was killed twenty-eight days, and dog no. 4 twenty-four days after operation. No gross changes were observed at

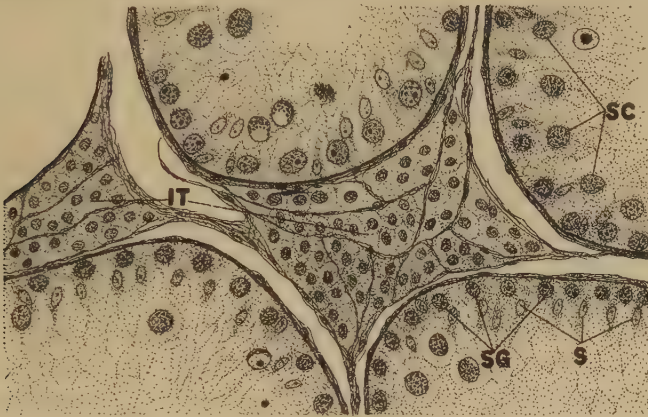


Fig. 2 Area of a section of the right testis of dog no. 2. *IT.*, interstitial tissue; *S.*, nuclei of sustentacular cells; *SC.*, spermatocytes; *SG.*, spermatogonia.

autopsy in either case, except that the right spermatic cord was congested and the right testis was somewhat hyperaemic.

Preparations of the left testis, in both cases, appear perfectly normal. Preparations of the right testis, in both cases, show initial degenerative changes in the epithelium of the seminiferous tubules. In some of the tubules spermatogenesis has ceased and extensive degeneration of the epithelial cells is apparent. Cytoplasmic changes are also apparent in the deeper layers of the epithelium, but a distinct degeneration reticulum is not yet present. In the majority of the seminiferous tubules spermatogenesis has not yet ceased. In many of them sloughing of epithelial cells has begun, but in others degenerative changes are

not yet apparent. The interstitial tissue has undergone no apparent change.

A comparative study of preparations of the right testes of these dogs and those of dogs nos. 1 and 2 shows conclusively that, although the former dogs were allowed to live as long after operation as the latter, the degenerative changes following the elimination of the sympathetic nerve supply to the testis, though identical in character, were initiated later and progressed less rapidly in the animals in which the left testis was removed at the time of operation than in those in which both testes were left in situ.

CHANGES FOLLOWING LIGATURE AND RESECTION OF THE RIGHT DUCTUS DEFERENS

Dog no. 5 was subjected to operation on April 22nd. The right ductus deferens was ligatured and resected through an abdominal incision. This dog was killed on May 13th, i.e., twenty-one days after operation. No gross changes in the testes were observed at autopsy.

Sections of the right testis show well-marked degenerative changes in the seminal epithelium. Spermatogenesis has practically ceased throughout the entire gland. Spermatozoa with their heads imbedded in the cytoplasm of the sustentacular cells are still present in small numbers in some of the seminiferous tubules. All the seminiferous tubules show extensive sloughing of epithelial cells which involves primarily the spermatids and spermatocytes. Many of these cells slough before necrotic changes are apparent. The cells which remain in situ in the seminal epithelium show little or no evidence of degenerative changes. The interstitial connective tissue has undergone no apparent change. The interstitial secretory tissue is somewhat hypertrophied.

Sections of the left testis show degenerative changes in the seminal epithelium identical in character with those which have occurred in the right testis, though somewhat less advanced. Spermatogenic activity is materially reduced throughout the entire gland. In many of the seminiferous tubules it has appar-

ently ceased. Nearly all of the seminiferous tubules show more or less extensive sloughing of epithelial cells which involves primarily the spermatids and spermatocytes. No apparent changes have occurred in the interstitial connective tissue. The interstitial secretory tissue has probably undergone slight hypertrophy; however, the evidence on this point is not conclusive.

Dog no. 6 was subjected to the same operation as dog no. 5, but under local anesthesia. This dog was killed thirty days after operation. No gross changes in the testes were observed at autopsy.

Preparations of the right testis show advanced degenerative changes. Spermatogenesis has ceased. No spermatozoa are present in any of the seminiferous tubules or any of the efferent ducts. The epithelium of the ductus epididymis is distinctly vacuolated. In many of the seminiferous tubules all of the spermatogenic cells except a relatively small number of spermatogonia have disappeared and a relatively coarse degeneration reticulum is present throughout the epithelium. The nuclei of the spermatogonia which remain in situ appear quite normal. The nuclei of the sustentacular cells also have undergone no apparent change. The interstitial secretory tissue is markedly increased throughout the entire gland and in many areas of the sections shows evidence of active hyperplasia.

Preparations of the left testis show degenerative changes in the seminal epithelium and in the epithelium of the ductus epididymis which are identical in character with those in the right testis, but slightly less advanced. They also show corresponding hypertrophy of the interstitial secretory tissue. Figures 3 and 4 illustrate portions of the walls of seminiferous tubules in areas of sections of the right and left testis, respectively, in which the degenerative changes are well advanced.

The degenerative changes observed in the left testis in these two cases were quite unexpected. Inasmuch as they are identical in character with the changes in the right testis following ligature and resection of the right ductus deferens, they can hardly be interpreted as the results of anesthesia or shock due to operation. However, in order to eliminate these factors as far as possible,

dogs nos. 7 and 8 were subjected to an operation, without anesthesia, in which the right ductus deferens was ligatured and resected through a superficial incision just proximal to the scrotum. These dogs were killed at intervals of twenty and twenty-nine days, respectively, following operation. No gross changes in the testes were observed at autopsy in either case.

Microscopic examination shows degenerative changes in both the right and the left testis of both dogs. In the case of dog no. 7 spermatogenesis has ceased in the right and has become materially reduced in the left testis. Extensive sloughing of

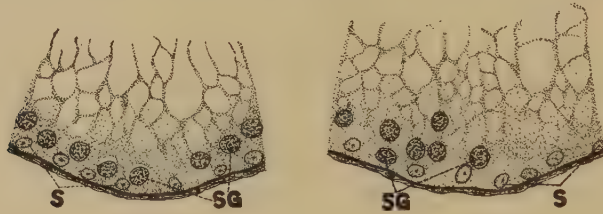


Fig. 3 Section of the wall of a seminiferous tubule in the right testis of dog no. 6. *S.*, nuclei of sustentacular cells; *SG.*, spermatogonia.

Fig. 4 Section of the wall of a seminiferous tubule in the left testis of dog no. 6. *S.*, nuclei of sustentacular cells; *SG.*, spermatogonia.

epithelial cells is apparent in many of the seminiferous tubules and the epithelium of the ductus epididymis is becoming vacuolated in both testes. Spermatozoa are still present in small numbers in the right testis in a few of the best-preserved tubules, while in others sloughing has advanced so far that but few spermatogenic cells remain in situ. In the left testis spermatozoa are still present in many of the seminiferous tubules, although the epithelium of these tubules shows evidence of degeneration. In other tubules sloughing has advanced almost as far as in the tubules of the right testis. The epithelium of the ductus epididymis is distinctly necrotic in both testes. The interstitial secretory tissue in these testes is somewhat hypertrophied; however, the increase in the quantity of this tissue is not well marked.

A comparative microscopic study of the testes of dogs nos. 5 and 6 and of dogs nos. 7 and 8 shows that in the latter as well as in the former the seminal epithelium both in the right and the left testis has undergone degenerative changes which are identical in character; however, the degenerative processes have advanced less rapidly in the latter than in the former. Hypertrophy of the interstitial secretory tissue has accompanied degeneration of the seminal epithelium in each case in which such degeneration is well advanced.

DISCUSSION

A comparative study of the degenerative changes in the seminal epithelium in the testes of the two series of dogs used in this investigation shows clearly that degeneration of the seminal epithelium following elimination of the sympathetic nerve supply to the testis is not identical with the degeneration of this tissue following ligature and resection of the ductus deferens. Degeneration due to the former cause involves first the spermatocytes and spermatids. These cells become necrotic in situ and gradually slough. In most instances the last of the spermatids disappear from the tubule, while the spermatocytes are still relatively numerous in the deeper layers of the epithelium. As the degenerative changes progress both the spermatogonia and the sustentacular cells also become involved. The cytoplasm of the latter becomes involved relatively early, but changes in the nuclei of these cells are not very apparent until the spermatogonia have become materially reduced in number. Degeneration due to ligature of the ductus deferens also involves first the spermatocytes and spermatids; however, sloughing is a more important factor in degeneration due to this than to the former cause. Many of the cells slough before they appear necrotic. In many instances the great majority of the spermatids and spermatocytes in the superficial layers of the epithelium have sloughed before well-marked degenerative changes are apparent in any of the cells in the deeper layers. In many of the tubules nearly all of the spermatocytes have sloughed before any material reduction in the number of spermatogonia becomes apparent.

By reason of the extensive sloughing, the degeneration reticulum which appears in all of the seminiferous tubules as degeneration advances is less extensive than in testes in which degeneration has resulted from the former cause.

Degeneration of the seminal epithelium due to ligature or occlusion of the ductus deferens has been described in various mammals. It is very characteristic in type and differs in several particulars from degeneration of this tissue due to other causes. The degeneration of the seminal epithelium following the elimination of the sympathetic nerve supply to the testis described above is very similar in type to degeneration of this tissue due to certain other causes. Regaud ('10) described degeneration of essentially the same type in the seminal epithelium in the testes of mice, rats, guinea-pigs, rabbits, and cats. The cause of this degeneration is not stated. Barratt and Arnold ('11) described degeneration of the same type in mammalian testes which had been subjected to x-rays. Very similar degenerative changes in the seminal epithelium were described recently by Allen ('19) in the testes of albino rats. Allen's observations were made on the testes of two series of rats. In one series the degenerative changes are due to a diet deficient in the water-soluble vitamine. The other series consisted of ten rats which, he says, "were all subjected to a reduced diet for fifteen days, beginning when they were ten weeks of age, while half of them were also subjected to the fumes of alcohol for a period long enough to produce complete intoxication daily during a time varying from seventy-seven to one hundred and fifty-five days." My observations regarding the type and order of degeneration agree in general with those of the authors here cited. They are not in accord with those of Barratt and Arnold, which, they claim, indicate an increase in the number of sustentacular nuclei in the final as compared with the earlier stages of degeneration, but agree fully with those of Allen, which indicate a reduction in the number of these nuclei in the later stages of degeneration.

Hypertrophy of the interstitial secretory tissue in the testis following exposure to x-rays was reported by Bergonie and Tribondeau ('04). A marked increase in the quantity of the

interstitial tissue was also observed by Allen in the testes of the rats which were fed on a diet deficient in the water-soluble vitamine. My own observations indicate marked hypertrophy of the interstitial tissue in all of the testes in which degeneration of the seminal epithelium is well advanced. They are distinctly at variance with the statement of Bouin and Ancel that resection of the nerves in the spermatic cord alone may cause atrophy of the interstitial tissue in the testis.

In dogs nos. 1 and 2, as observed above, the left testis shows degeneration of the seminal epithelium identical in character and almost or quite as advanced at the time of autopsy as the right testis. It also shows a corresponding hypertrophy of the interstitial secretory tissue. Degeneration in the testes of these dogs followed the removal of the inferior mesenteric ganglia and resection of the nerves descending along the right spermatic artery. As was pointed out in the foregoing paper,² the seminal epithelium receives no sympathetic nerve supply. Its degeneration in these dogs is probably the result of nutritive disturbances due to the paralysis of the blood-vessels in the spermatic cord and testis. Nevertheless, degeneration in the left testis, in these cases, suggested the conclusion that the removal of the inferior mesenteric ganglia eliminates the essential nerve supply to the testes. This conclusion is probably correct; however, in the light of the results following ligature and resection of the ductus deferens on the right side only, such a conclusion is not justified by a study of degenerative changes alone. In the dogs in which the right ductus deferens was ligatured and resected, the left testis also showed degenerative changes in the seminal epithelium identical in character with the degenerative changes in the seminal epithelium in the right testis. It also shows corresponding hypertrophy of the interstitial secretory tissue. Inasmuch as degeneration of the seminal epithelium due to ligature or occlusion of the ductus deferens differs somewhat from degeneration due to other causes referred to in this paper, and inasmuch as two of these dogs were operated without anesthesia, we cannot conclude that degeneration in the left testis, in these cases, is the

² The innervation of the gonads in the dog. *Anat. Rec.*, vol. 17, pp. 203-219.

result of shock, anesthesia, or accident. Obviously, it is associated with and conditioned by degeneration in the right testis. Whether the seminal epithelium in the left testis in cases of this kind would ultimately be restored remains to be determined.

As was observed above, in dogs nos. 3 and 4, which were subjected to the same operation as dogs nos. 1 and 2, except that in the former the left testis was removed at the time of operation, degenerative changes in the right testis were initiated later and apparently progressed less rapidly than in the dogs in which both testes were left in situ. Inasmuch as hypertrophy of the interstitial secretory tissue was not apparent in the right testis in either case, although the dogs were allowed to live twenty-eight and twenty-four days, respectively, following operation, we may conclude that hypertrophy of the interstitial secretory tissue is initiated earlier as an accompaniment of degeneration of the seminal epithelium than as a compensatory process due to the removal of one of the testes, if indeed such compensatory hypertrophy may occur.

In discussing the internal secretory function of the testis, Biedl ('13) cites the statement of Bouin and Ancel that in rabbits, six months after unilateral castration and ligature of the ductus deferens of the opposite side, the testis left in situ showed advanced degeneration of the seminal epithelium and enormous hypertrophy with signs of secretory hyperactivity of the interstitial secretory tissue. Biedl apparently concurs in the opinion of Bouin and Ancel that this hypertrophy of the interstitial secretory tissue is a compensatory process due to the removal of the other testis. In view of the fact that hypertrophy of the interstitial secretory tissue is a common accompaniment of degeneration of the seminal epithelium and in the light of the observation recorded above that in the two dogs in which the left testis was removed at the time of operation hypertrophy of the interstitial secretory tissue was not apparent in the right testis even after degenerative changes in the seminal epithelium were initiated, the opinion above cited is not well founded.

Neither is the conclusion justified that in cases of unilateral cryptorchidism in which the normal testis has been removed

before puberty hypertrophy of the interstitial tissue in the cryptorchidic testis is due to the absence of the normal testis. Cryptorchidic testes commonly show defective development or degeneration of the seminiferous tubules and complete absence of spermatogenesis, although the sustentacular cells may be well preserved. In view of the results of experimental work cited above, it is highly probable that hypertrophy of the interstitial tissue in cryptorchidic testes is an accompaniment of degeneration of the seminal epithelium.

SUMMARY

Elimination of the sympathetic nerve supply to the testis is followed by degeneration of the seminal epithelium and accompanying hypertrophy of the interstitial secretory tissue. This degeneration is similar in character to degeneration of the seminal epithelium due to certain other causes, viz., exposure of the testis to x-rays, a diet deficient in the water-soluble vitamine, etc.

Degeneration of the seminal epithelium, due to the same cause, and associated hypertrophy of the interstitial secretory tissue were initiated later and progressed less rapidly in dogs in which the left testis was removed than in dogs in which both testes were left in situ. Therefore, hypertrophy of the interstitial secretory tissue is probably initiated more promptly as an accompaniment of degeneration of the seminal epithelium than as a compensatory process due to the removal of one of the testes, if indeed such compensatory hypertrophy may occur.

Ligature and resection of the right ductus deferens in four dogs was followed by degeneration of the seminal epithelium and accompanying hypertrophy of the interstitial secretory tissue in both testes. Degeneration in the left testis in these cases is, doubtless, associated with and conditioned by degeneration in the right testis.

LITERATURE CITED

- ALLEN, E. 1919 Degeneration in the albino rat testis due to a diet deficient in the water-soluble vitamine, with a comparison of similar degeneration in rats differently treated, and a consideration of the Sertoli tissue. *Anat. Rec.*, vol. 16, pp. 93-117.
- BARRATT, W., AND ARNOLD, G. 1911 Cell changes in the testis due to x-rays. *Arch. f. Zellforschung*, Bd. 7.
- BERGONIE ET TRIBONDEAU 1904 Action des rayons x sur le testicule du rat blanc. *Comp. Rend. Soc. de Biol.*, T. 2.
- BIEDL, A. 1913 Internal secretory organs. New York, 1913.
- KUNTZ, A. 1919 The innervation of the gonads in the dog. *Anat. Rec.*, vol. 17, pp. 203-219.
- RÉGAUD, C. 1910 Particularité d'actions des rayons de Röntgen sur l'épithélium séminal du chat. *Comp. Rend. Soc. de Biol.*, T. 68.

Resumen por el autor, R. J. Terry.

La relación del nervio facial con la cápsula ótica.

El esqueleto óseo del oído de los mamíferos está atravesado más o menos extensamente por el canal del nervio facial a su salida del cráneo. La cápsula ótica cartilaginosa de los embriones de los mamíferos está también atravesada por el nervio facial. Los estudios sobre el desarrollo de la cápsula ótica en los mamíferos han suministrado pruebas de la existencia de, por lo menos, dos elementos diferentes que entran en su constitución: uno de ellos es la pared cartilaginosa que encierra al canal coclear epitelial y los conductos semicirculares, el otro la comisura suprafacial, que es probablemente un derivado del parietal. Entre estos elementos está situado el canal para el nervio facial, en posición tal que la cápsula ótica propiamente dicha es posterior a él, y la comisura suprafacial, anterior. En los embriones de los reptiles el nervio facial pasa hacia la cabeza, a partir de la cápsula ótica, a su salida del condrocráneo por un orificio situado entre la cápsula ótica y una porción del piso o pared lateral del cráneo es decir, una parte parietal. El desarrollo de la cápsula ótica de los anfibios susministra pruebas de su origen independiente y fusión secundaria con porciones parietales y de la inclusión de aquellas porciones del séptimo nervio que salen del cráneo del adulto a través de la cápsula ótica. La posición del orificio de salida del nervio facial entre la cápsula ótica y alguna de las porciones parietales de la vida embrionaria es probablemente una relación común a los mamíferos, reptiles y anfibios, no manifestándose en la vida del adulto a causa de la fusión de los elementos cartilaginosos originarios y la osificación que sigue.

Translation by José F. Nonidez
Carnegie Institution of Washington

THE RELATION OF THE FACIAL NERVE AND OTIC CAPSULE

ROBERT J. TERRY

Washington University School of Medicine

FOUR FIGURES

The following observations, made in the course of different studies of the cranium, are believed to indicate that the typical position of the facial nerve at its exit from the chondrocranium is external of the otic capsule. This relation has been accepted for reptiles (turtle and lizard) in which the nerve passes out of the chondrocranium between the ear capsule and the praefacial commissure. In the case of amphibians and mammals, however, the nerve, or some part of it, is commonly described as traversing the otic capsule; in the former by a foramen in the floor or wall of the capsule, in the latter by a short canal extending from the internal acoustic meatus, beneath the suprafacial commissure. I shall endeavor to show that this commissure must be regarded as a derivative of the cranial wall, in part at least, and that the exit of the hyomandibular palatine branches of the facial nerve in amphibia is between the ear capsule and the floor of the skull (parachordal plate).

In adult *Necturus*, a foramen in the ventral surface of the skull gives exit to the facial nerve. This foramen is the outer opening of the stylomastoid canal which, as described by Wilder¹ "enters the pre-otic at its inner margin, traverses almost its entire ventral wall." The ramus palatinus passes through a foramen separate from that of the rest of the facial nerve. The foramina in the floor of the otic capsule for the exit of the ramus hyomandibularis and ramus palatinus of the facial are mentioned

¹ Wilder, H. H. 1903. The skeletal system of *Necturus maculatus* Rafinesque. Mem. Bost. Soc. Nat. Hist., vol. 5.

by Miss Platt² in the description of the skull of *Necturus* of 19 mm. These divisions of the facial accompany the auditory into the capsule, entering the latter from the cranial cavity by the large opening in the median wall of the capsule. Thus, in accordance with the foregoing statement, the facial nerve, or its subdivisions, traverses some part of the skeleton of the ear in passing out of the cranium. As to this being the true relation of facial nerve and pro-otic bone no question is raised, but as to the passage of the nerve through the cartilaginous otic capsule, the early history of this part of the cranium throws considerable doubt.

The auditory capsule of *Necturus* arises independently and is secondarily united with the basal plate by what Miss Platt has termed connecting bars. The union takes place in embryos of 18 mm., one connecting bar being formed just behind the spot where the ramus hyomandibularis leaves the facial ganglion and uniting the capsule with the trabecular part of the basal plate; a second connection being established in front of the facial ganglion, in consequence of which the hyomandibular and palatine branches pass out through foramina in the floor of the skull. My own observation is that the exit for both branches is first by a single, narrow, transverse opening which later is partitioned into the two definitive foramina. Miss Platt found that the floor of the auditory capsule is periotic in origin and not derived from the parachordal of the floor of the cranium. Additional evidence in support of this conclusion presents itself even in advanced stages of development. The conditions represented in figures 1 and 2 show quite clearly the limits of the otic capsule and parachordal in embryos of 22.5 mm. and 28 mm., respectively. In the former (fig. 1) the section passes through the facial ganglion and the combined rami hyomandibularis and palatinus at their exit from the skull. This opening stands between the basal (parachordal) plate and floor of the otic capsule, respectively. Upon the lateral side of the ganglion and within the otic capsule is the anterior division of the acoustic

² Platt, J. B. 1897. The development of the cartilaginous skull and of the branchial and hypoglossal musculature in *Necturus*. *Morph. Jahrb.*, Bd. 25.

nerve. In figure 2 the section passes through the facial ganglion just behind the exit of the hyomandibular and palatine branches. At this level the floor of the otic capsule overlaps the edge of the basal (parachordal) plate and is therefore easily differentiated from it. In view of these relations of ear capsule and cranial

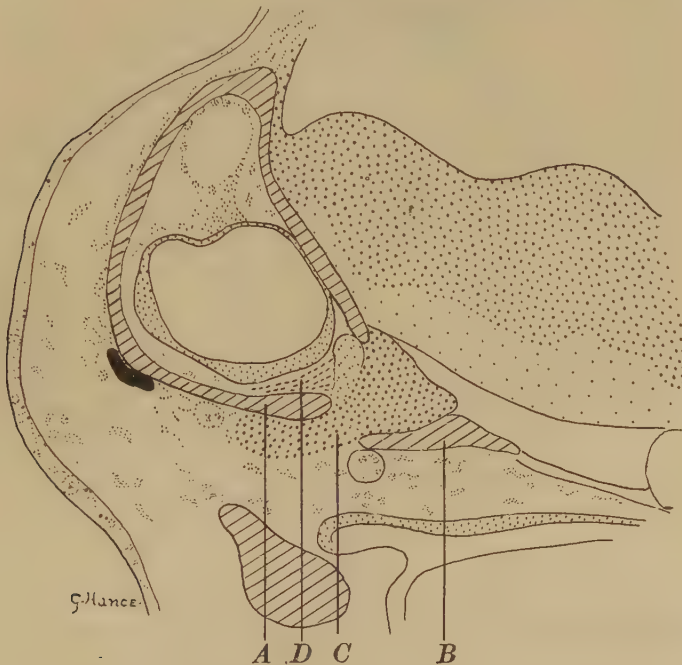


Fig. 1 *Necturus*; length 22.5 mm. Section 105 of series 135 W. A. C., passing transversely through the cephalic pole of the otic capsule at the level of the facial ganglion. *A*, floor of otic capsule; *B*, parachordal plate; *C*, ramus hyomandibularis and palatinus; *D*, anterior ramus of acoustic nerve. $\times 65$.

floor, it is evident that in embryos of *Necturus* the hyomandibular and palatine branches of the facial nerve do not traverse the otic capsule in leaving the skull, but pass between it and the lateral margin of the parachordal plate.

The great vacuity of the medial otic wall of young larvae is reduced at the stage of 46 mm. by growth and partitioning to four openings, as described by Miss Platt. Through the anterior

of these foramina pass the facial and anterior divisions of the auditory nerve. A secondary floor, derived from the ventral part of the anterolateral wall of the ear capsule grows inward to separate the hyomandibular part of the facial ganglion from the

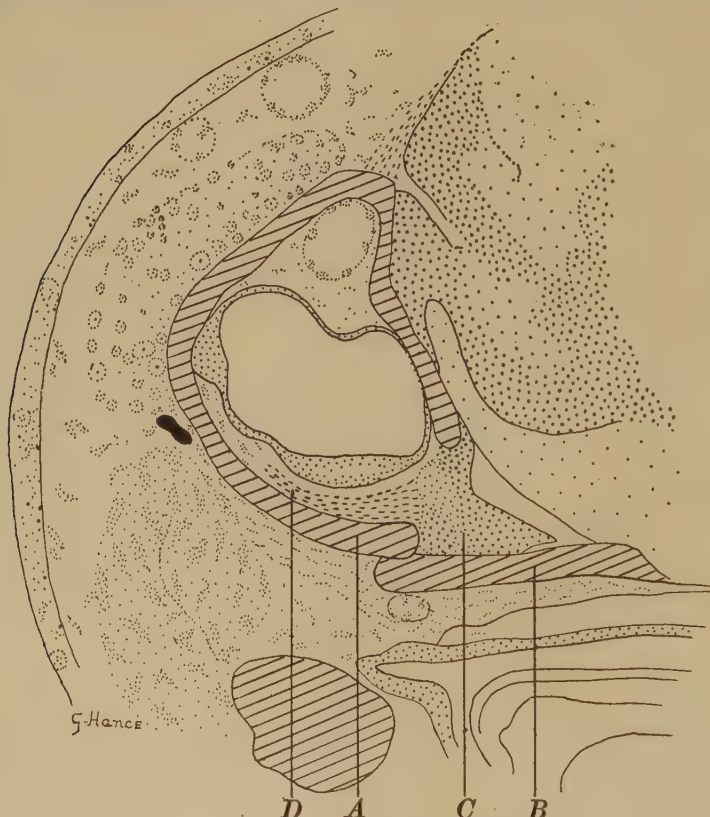


Fig. 2 *Necturus*; length 28 mm. Section 133 of series 137 W. A. C., passing transversely through otic capsule just caudad of level of exit of the hyomandibular and palatine rami of the facial nerve. Lettering same as in figure 1. $\times 65$.

auditory chamber. This secondary floor is, I believe, the proper floor of the otic capsule just completed at the stage of 46 mm. I wish to emphasize the fact that the formation of this ventral part of the capsule still leaves the facial nerve in its original extra-capsular relation which obtains in younger stages (fig. 3).

Although I have not examined the skulls of other Amphibians with the object of determining the relation of facial nerve and otic capsule, it seems highly probable from descriptions of several

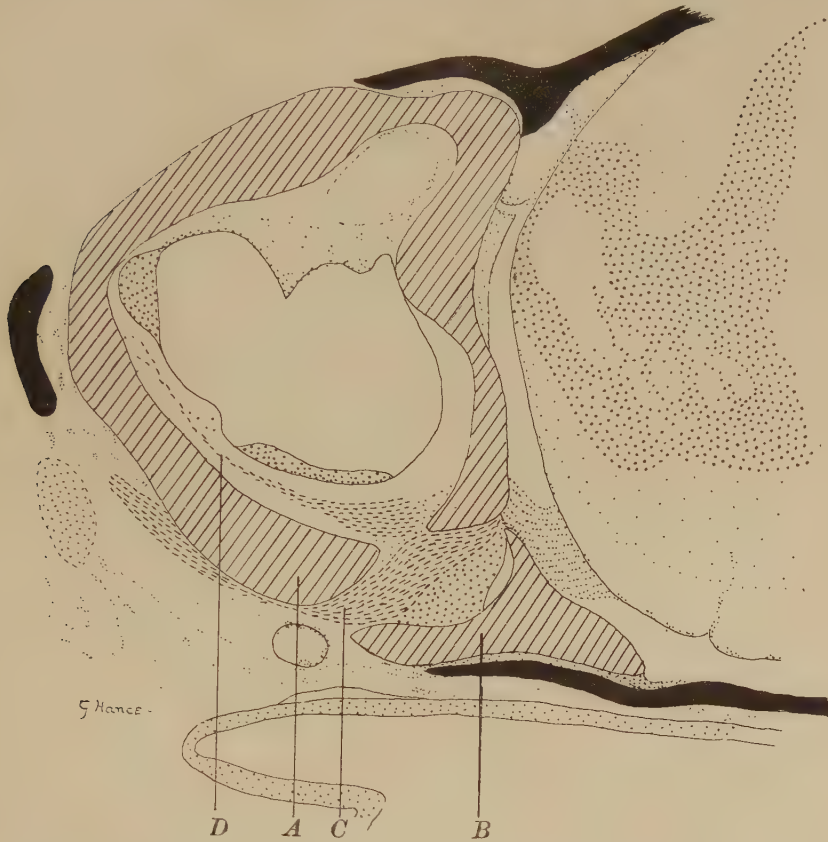


Fig. 3 *Necturus*; length 49.5 mm. Section (transverse) 113 of series 139 W. A. C. through the otic capsule at the level of the exit of the hyomandibular and palatine branches of the facial nerve. Lettering as in figure 1. Circa 65.

species that the course of the nerve is extracapsular as in *Necturus*. Winslow's³ account of the skull in larvae of *Amblystoma* (stages of 11 and 12 mm.) supports this belief. The periotic

³ Winslow, Guy M. 1898. The chondrocranium in the Ichthyopsida. Tufts College Studies, no. 5.

capsule arises independently in this amphibian and in uniting with the basal plate apparently establishes relations similar to those in *Necturus*; that is, the hyomandibular nerve apparently makes its exit between the floor of the cranium and the ventral wall of the otic capsule.

In the turtle the facial nerve passes out of the chondrocranium through a foramen formed by the otic capsule and the cartilaginous bar called praefacial commissure. In *Emys*, Kunkel⁴ found the facial foramen situated at the juncture of the basal plate and otic capsule, anterior to the cochlea; the praefacial commissure extending from the basal plate to the anterior cupola of the otic capsule. This also is essentially the condition in *Chrysemys* as I have observed. As to the origin of the praefacial commissure I have no reliable data: in an embryo of *Chrysemys* of 8.5 mm. the chondrification of the commissure next the otic capsule was not so far advanced as in the rest of its extent, but this observation is without confirmation.

The manner of exit of the facial nerve from the chondrocranium of mammals has been described by a number of authors: in company with the anterior division of the acoustic nerve the facial enters a shallow internal acoustic meatus of the otic capsule, from which it passes into a short facial canal or foramen covered by the suprafacial commissure to emerge outside the chondrocranium.

The suprafacial commissure, unlike the praefacial commissure of the turtle, apparently has no relation to the basal plate; it has been described by writers as connected with the ear capsule only. However, certain conditions, described in a previous paper (Terry⁵) incline me to believe that the suprafacial commissure of mammals is in part, at least, a parietal structure which has become incorporated with the periotic capsule, and that therefore the facial foramen is a passageway for the nerve, not through the periotic capsule, but between it and a part of the cranial wall. These conditions will be briefly related.

⁴ Kunkel, B. W. 1912. The development of the skull of *Emys lutaria*. Jour. Morph., vol. 23.

⁵ Terry, R. J. 1917. The primordial cranium of the cat. Jour. Morph., vol. 29.

In cat embryos of 15 mm. I have found continuity of the suprafacial and orbitoparietal commissures when, at the same time, the latter is demarcated from the otic capsule by a plane of mesenchyma. This plane is the meeting place of the independently arising otic capsule and the commissure. By the disappearance later through chondrification of this as well as other

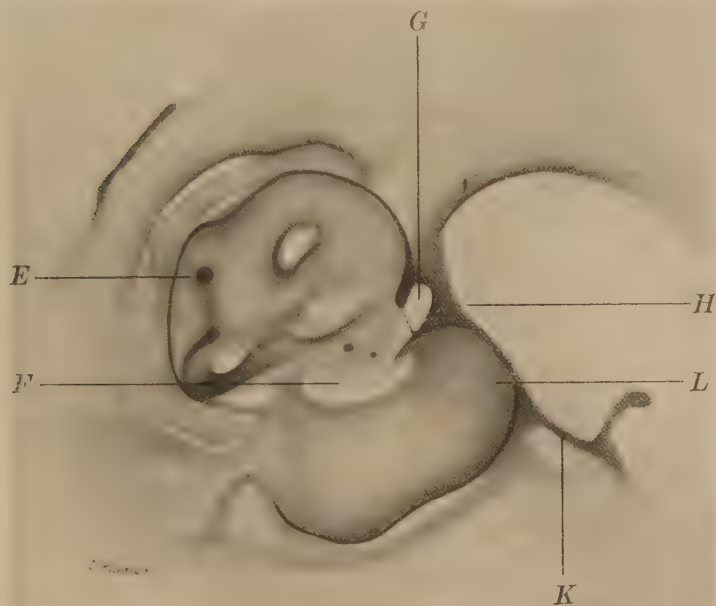


Fig. 4 Cat; length 30 mm. W. A. C. no. 390. Medial aspect of left otic capsule as it appears in a van Wijhe preparation. *E*, pars canicularis; *F*, int. acoustic meatus; *G*, facial foramen; *H*, suprafacial commissure; *K*, aliochlear commissure; *L*, pars cochlearis. Circa 14.

otic boundary zones, the last evidence of the original state of independence of the ear capsule is lost. Consequently, it is impossible in older embryos to define the original relations of the suprafacial commissure: it seems to be, as commonly described, only a part of the otic capsule. In cat embryos of 24 to 30 mm. the continuity between the suprafacial commissure and the orbitoparietal commissure, as revealed by van Wijhe preparations, is clearly shown (fig. 4). The figure shows the

commissure as it appears in the preparation to be in the form of a slender arch stretching from the roof of the cochlea high over the facial nerve to the commissura orbitoparietalis and prominentia utriculo-ampullaris. In following the commissure medially some indication of an indirect relation with the base of the skull was found in embryos of 12-mm. length. This relation was not very clearly seen in several specimens examined, whereas in others continuation of the tissue of the suprafacial commissure was observed as far as the level of the floor of the skull.

Chiefly on account of the growth of the suprafacial commissure, a meatus acusticus internus is formed, as I have shown in another place, resulting in a closer relation of the facial and acoustic nerves and apparently burying the former in the otic capsule. The original extracapsular relation is nevertheless still preserved, the nerve passing out of the cranium through a foramen between the otic capsule and the suprafacial commissure.

Resumen por el autor, Raphael Isaacs,
Universidad de Cincinnati.

La estructura y mecanismo del desarrollo del tejido conectivo.

Cuando se precipita plasma y linfa, el coágulo de fibrina resultante presenta una estructura bien desarrollada en forma de fibrillas entrecruzadas. El origen de tal estructura definida a expensas de un líquido indica que las estructuras fibrilares descritas en otros tejidos pueden tener un origen semejante. Tales texturas fibrilares pueden observarse en los tejidos conectivos, membranas basales, substancia de cemento intercelular y en los tejidos de neuroglia del sistema nervioso, después de la fijación de dichos tejidos. Estas fibrillas no son visibles en el tejido vivo, pero aparecen claramente cuando se fija, deshidrata o calienta. El proceso puede estudiarse al microscopio y difiere con los varios fijadores. Los jugos extraídos de los tejidos, filtrados y fijados originan imágenes que recuerdan las fibrillas que aparecen en los tejidos fijados. Los métodos de digestión no demuestran las fibrillas hasta que alguna de las manipulaciones de la técnica implica un proceso de coagulación o deshidratación. Las células del tejido fijado exhiben los efectos de la presión pero las "fibrillas" no presentan tales efectos. Las fibras del tejido adulto se forman por el engrosamiento de la gelatina homogénea que existe entre los fibroblastos. Cuando se tratan por fijadores, las fibras jóvenes adoptan a menudo la estructura de una red. Como esta red se hace más gruesa por aumento en la concentración de la gelatina, tal proceso ha sido descrito por algunos autores como la transformación de una red en fibras. Para un coloide determinado, dentro de ciertos límites, cuánto más diluída está la "solución", más ligero es el precipitado fibrilar. Esto suministra un método para el estudio de los tejidos fijados e indica un determinismo fisiológico en el crecimiento de los capilares.

Translation by José F. Nonidez
Carnegie Institution of Washington

THE STRUCTURE AND MECHANICS OF DEVELOPING CONNECTIVE TISSUE

RAPHAEL ISAACS

College of Medicine, University of Cincinnati

SIX FIGURES

When the fluid part of blood is precipitated, the clot of fibrin has a well-developed structure of interlacing fibrils. The production of this definite structure from a fluid suggests that fibrillar appearances elsewhere in the tissues may have a similar origin. Such fibrillar textures are seen in connective tissue, in basement membranes, in cement substance between cells, and in the neuroglial tissue of the nervous system. The present paper is a study of these structures and deals with the growth, consistency, and reactions of connective tissue and cement substance. The conclusions point to the view that the so-called connective-tissue fibrils are artifacts, and that the cement substance and basement membranes are parts of a homogeneous intercellular jelly. The variation in precipitation pattern gives a histological basis for recognizing different stages in the physiology of organs.

NOMENCLATURE

In a histological section of 'fixed' connective tissue, fine fibrils can be seen stretching between the cells (fig. 1). These are called connective-tissue fibrils (Mall, '02) or exoplasmic fibrils (Mall, '02; Flint, '04) or collagenous fibrillae (Bell, '09). In the central nervous system a somewhat similar group of fibrils are known as neuroglia fibrils or fibrillated endoplasm (Hardesty, '04). The name white or collagen fibers is given to a group of highly refractive, homogeneous strands of tissue found in skin and tendon, as well as in other parts of organs. The yellow or elastic fibers are also definite large threads of tissue, found

in many organs. This paper deals with the development of the white and yellow fibers, and also the intercellular jelly, a homogeneous substance lying between the cells and fibers, and giving rise, according to this view, to artificial fibrils on fixation or dehydration.



Fig. 1 Appearance of connective-tissue fibrils and fibrin clot in vessels. Photomicrograph. 55-mm. pig embryo. Bouin. Mallory's connective-tissue stain and iron haematoxylin.

MATERIALS AND METHODS

For the purpose of studying the structure and development of connective tissue, chick, pig, and human embryonic material of different ages was used, the fixation and staining being varied to study the effects under various conditions. Living tadpoles and embryos of the chick and pig and adult frogs were used for the study of fresh tissue. The experiments were conducted along two lines. The nature of the tissues was studied from the animal tissue, and experiments were carried out with colloid solutions of gelatin, egg albumin, and fibrin, of known strength and composition, under controlled laboratory conditions. The technique in each case is given under the discussion of the phenomena in question.

BEHAVIOR OF CERTAIN COLLOIDS

In dealing with living tissues, we are studying substances in a colloid state. Some of the properties of protoplasm are properties of colloids. When we see protoplasm absorbing water or secreting it, we are naturally reminded of a similar behavior in such substances as gelatin, fibrin, or white of egg. In these substances we can, by using filtered solutions, free them from morphological structures. Yet on precipitation we can produce elaborate patterns (Hardy, '99; Butschli, '92) (fig. 2, C). These substances, when in the jelly state, can give rise to structures, resembling fibers and fibrils, if they are put under pressure or stress. A gelatin jelly, on pressure, can be broken into many droplets of different sizes, which give rise to structures resembling fibers and other details of tissues. These structures round up into drops when pressure is released. The behavior of fresh connective tissue is much the same. When compressed between cover-glasses under the microscope, we see many structures, but release of pressure results in little gelatinous droplets with but little structure.

Syneresis, the property of colloids which gives rise to the secretion of a fluid containing the substance of the colloid in a dilute state, must be taken into consideration when the colloids

of the tissues are considered. This process takes place comparatively quickly when viewed under the microscope, and a few minutes make a definite change in the consistency, toughness, and refraction of the colloid studied. The colloids which tend



Fig. 2 Corresponding areas of subcutaneous connective tissue of a six-day chick, fixed with various solutions and stained with Mallory's connective-tissue stain.

A. Connective tissue extract fourteen-day chick (salt solution), filtered, precipitated with Zenker's solution and stained with Mallory's connective-tissue stain. Camera-lucida drawing.

B. Fixed in Bouin's solution.

C. Egg albumin, filtered, and precipitated with Zenker's solution. Camera-lucida drawing.

D. Fixed in Zenker's solution.

E. Fixed in Van Gehuchten's solution.

F. Fixed in absolute alcohol. Camera-lucida drawings.

to undergo irreversible changes, as white of egg, show this property to a marked degree, and the differences in appearance are striking. One does not appreciate what elaborate structures and quick changes can be produced in this way until the process is studied under a high magnification. The structures produced can be emphasized by stains.

Many inorganic salts, when precipitated under the microscope, present 'patterns' of interlacing fibrils, composed of strings of minute granules or crystals. Such pictures simulate the fibril patterns of colloidal proteins, and remind one of the délicaté cytological structures often shown in fixed tissue.

THE INTERCELLULAR JELLY

The jelly-like nature of young embryos is a matter of common experience with all who have handled young chicks or pigs. When lifted up by any part, they elongate and tend to stretch. They have the consistency of thick mucus, and a very small force is required to tear off a part or cause compression or strain. With increase of age, an increase of firmness is noted. For microscopical examination of the intercellular substance it is necessary to put small pieces in a hanging drop in a moist chamber or underneath a cover-glass on a slide, sealed with vaselin, no fluid of any kind being added. The temperature can be kept constant and evaporation can be avoided to a certain extent. However, the pulling and squeezing of the tissue in handling and cutting and the changes of tension when flattened against the cover-glass are factors to be considered in interpreting the results. Under favorable conditions, observations may be taken on the tissue for a few minutes without much physical change. Maximow ('06, p. 683) used a somewhat similar method, but as this technique did not show up certain cellular structures which he had expected, he emphasized these structures by producing a local oedema with physiological salt solution. The results of such a procedure, however, require cautious interpretation, as the equilibrium of the intercellular colloids is easily disturbed, a process often encountered in the physiology and pathology of connective tissue.

The subcutaneous tissue in a five-day chick reacts as a mass of jelly when touched. The tissue can be indented with a blunt needle, and the cells and substances around the point are bent. If a piece of tissue be 'fixed' in this position, the position cells will show the results of the pressure, but the "connective tissue

fibrils" will radiate in all directions, independently of the lines of force of the pressure. If they had been present in the living tissue, one would expect to see some results of compression, as the cells themselves show. In the living, the cells and the substance between them act as if they were a mass of the same consistency throughout, and the physiological unit for response to pulls and tension is the region affected, not separate cells.

When tissue is mounted as described, it becomes flattened against the cover-glass, and a narrow zone of a jelly-like colloidal substance, containing granules, forms the peripheral region. This jelly responds to a touch with a needle, much as does the tissue itself. On indenting one side, granules throughout the jelly move in response to the strain set up. The jelly is probably composed of intercellular substance—"tissue juice," lymph, and plasma. The intercellular colloid is more viscous than lymph, and does not run up into a capillary tube, as does the latter.

Varying with the conditions, this jelly undergoes a change on standing from two to five minutes. The granules of various kinds begin to agglutinate around the outer edge of the jelly ring, and the peripheral zone becomes stiffer. A process resembling crystallization takes place, resulting in the formation of a network from the masses of granules. The network is microscopic, and under low power resembles a fuzzy mass with a ground-glass effect. The basis is a fine matrix of fibrillae, made up of granules, but sometimes it is fairly homogeneous. It resembles connective-tissue fibrils in appearance, taking the same stains—*aniline blue*, *orange-G*, and *acid fuchsin*. The behavior is similar to that of coagulating fibrin. Ranvier ('89) described a similar process as the normal method of formation of the large white fibers of the connective tissues.

This process may be hastened by drying, heat, dehydrating, and coagulating fixatives. Formalin gas produces a fairly homogeneous fixation, but dehydrating destroys this effect. The fibrils formed correspond closely to Baitsell's ('15) fibers, formed from the fibrin clot of cultures of chick tissue *in vitro*. He points out that "the transformation of the fibrin net results in the shrinkage of the clot. It also becomes very tough and resistant to

injury." The process is hastened by mechanical manipulation of the clot with needles. This same phenomenon can be reproduced in filtered egg albumin, manipulation giving rise to the appearance of well-defined fibrils. The intercellular substance clots as if it had fibrin as its basis, but the variation in staining and the consistency during life give the impression that some mucoid elements are present in addition. The jelly is more viscid than either plasma or lymph, and does not run, as do these fluids, but it can be made to undergo a gradual flowing. This holds true for all stages, from the embryonic to the adult tissue.

As the peripheral fibrils form in our preparations, a watery fluid accumulates just around the tissue itself and in the meshes of the fibrils. This process, in effect, is analogous to that of syneresis in colloid gels, and is familiar to us in the liquid accumulation over agar-agar or gelatin jelly. It takes place independently of drying effects (Graham in M. Fischer, '15, p. 240). As soon as this dilute liquid forms, the cells in contact with it swell, probably due to the increased acid content as the tissue dies or to the availability of 'free' water. This test, accompanied by the brighter appearance of the nuclei, which in the perfectly fresh tissue can be only indistinctly located, we use as signs of the beginning of the death process. The nuclei appear brighter, either because they undergo a change of consistency and become more viscous or else because the cytoplasm becomes less dense, due to the absorption of water. When the term fresh tissue is used in this paper, it refers to the condition before the appearance of these changes. The blood corpuscles do not change shape for some time after this, and appear less sensitive than the embryonic tissue cells in this respect. However, as the changes take place, the nuclei of the erythrocytes show very clearly in the chick material. The fact that blood plasma is relatively more dilute than the intercellular colloids probably accounts for this difference of behavior, and this factor should be taken into account in interpreting tissue cultures in which plasma is used. The preservation of the shape of the blood corpuscles is no test for isotonicity as far as the tissues are concerned, as the corpuscles do not change in salt solution in the presence of 'free' water (not

in colloid combination). The tissue cells under these circumstances are affected immediately.

In the fresh tissue itself (chick and pig embryos) the position of the cells can be made out fairly accurately. No free-flowing intercellular 'tissue lymph' can be demonstrated. On tilting a slide containing a tissue mount, the intercellular substance remains. It does not run out under pressure, showing that most of the liquid is held in colloid combination. Sufficient pressure, however, easily crushes the cells, and a considerable amount of liquid is liberated in this way. This liquid flows readily, differing from the intercellular colloid. The spaces, corresponding to the intercellular connective-tissue spaces of fixed tissue, are filled with a clear, homogeneous jelly-like substance, which, in the younger embryos, has the consistency (not necessarily concentration) of a 'wobbly' gelatin gel.

The phenomenon of compression of this colloidal material is very instructive, as we can easily reproduce some of the processes taking place in the developing embryo. A needle pushed into the tissue causes a response in all parts of the tissue, as seen by the movement of visible granules. It can be described best as the jarring of a colloid jelly. Cuts close up with but little evidence of separation, and the pathway of a needle withdrawn is apparently obliterated. If a piece of the tissue is suspended from the tip of a needle or forceps, the lower end rounds up, as does a drop of stringy mucus. If a freshly cut piece of tissue is placed on top of a second piece, and the two are killed and fixed in this position, with no other pressure than the weight of the tissue, it is found on sectioning that fibrils extend in places, without interruption from piece to piece (fig. 3). This suggests that the fibrils are formed by the dehydrating or coagulating action of the fixatives from the homogeneous jelly. If, however, the bridging fibrils were merely pre-existing fibrils of one piece which have stuck to the other piece, then we would expect the fibril to be present throughout the gap between the pieces of tissue. Fibrin would give a similar picture. The fibrils are present, however, only in places, presumably where the colloid has had time to ooze. Of course, air bubbles

must be excluded. Fibrils in sections, then, may stretch across between parts which in the living may have been in contact or separated by the intercellular jelly. Sections often show such pictures around the more solid organs, as the thyroid or thymus, and they suggest that these organs evidently push into the connective tissue, which conforms to the new, irregular outline, by

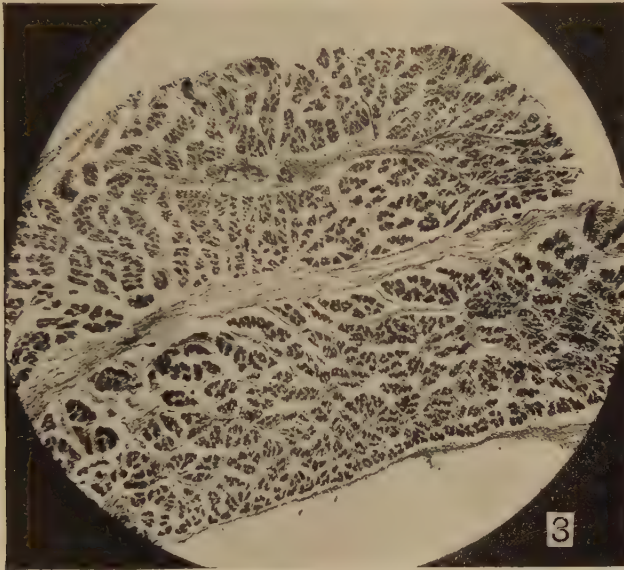


Fig. 3 Two pieces of tissue, which have been allowed to touch, and have been killed in this position. Photomicrograph. Mallory connective-tissue stain.

a flowing or oozing process, reminding one of the tissue closing in on the pathway of a withdrawn needle.

In compressed tissue, which is fixed and sectioned, the cells show the result of the pressure by their alignment—usually being flattened out, with their long axis perpendicular to the direction of the pressure—but the fibrils of the section show no evidence of the stress.

In living tadpoles Clark ('12) describes a delicate network of minute fibrillae between the cells. These, however, are not as numerous as the fibrils which the fixed tissue show. These

fibrils, which are seen in the living, can be picked out from the connective-tissue fibrillae after the section is fixed, and suggest the branching cytoplasmic processes of stellate cells.

When a precipitating agent, as mercuric chloride, acts on colloidal solutions, as of egg albumin, of different strengths, the substance is precipitated in greater bulk from the more concentrated solution, and therefore leaves a denser, more closely packed mass. In the weaker solutions the mass originally is much less dense, but when it settles, the mass may appear as dense as that from the thicker solution. However, in the weaker solutions it will be noted that the supernatant solution is often cloudy, turbid, or opalescent with a fine precipitate which does not tend to settle out. In the stronger solutions, this may be carried down with the rest of the flocculent precipitate, or else in the stronger solutions, the precipitated granules are larger. A. Fischer ('99) notes that the thinner the solution of a colloid, the smaller the granules precipitated with reagents.

If, then, a weaker, but not necessarily a less viscid solution of a colloid will leave less precipitate than a more concentrated one when thrown down, then the strength of colloidal solutions in tissues can be judged by the amount of residue they leave in fixed sections. The very young embryos show a much more semi-fluid condition when picked up than the older ones. Schäfer ('12, p. 116) points out that the albuminous substances of the cell interstices of very young embryos later acquire a muco-albuminous character, and the tissue assumes a jelly-like consistency. Triepel ('11) describes a corresponding series for fixed sections, a fine network in young stages, which becomes coarser as the embryo grows older.

On pressure on the subcutaneous connective tissues taken from a four- to seven-day chick mounted between a cover-glass and slide and sealed with vaselin, pieces can be made to separate off from the central mass, just as pieces can be broken off of a 'wobbly' gelatin gel. If the microscope is tilted, these pieces will slip down, accommodating their outline to the surrounding obstacles. Such a mass, on flowing between two fixed particles (as pieces of glass), will be drawn into a very narrow thread,

as a string of ropy mucus. On flowing through, it is reconstituted or regathered as a mass as soon as an open space is reached (fig. 4). The ease with which a group of cells separate and regather with little or no trace of their experience, even on fixation, suggests that the syncytial appearance of young connective-tissue cells is a temporary, apparent union of the cells, easily changed by the conditions of the environment. It is not impossible to imagine a similar process taking place on handling and fixing an embryo. Lymphatic vessels and capillaries may be

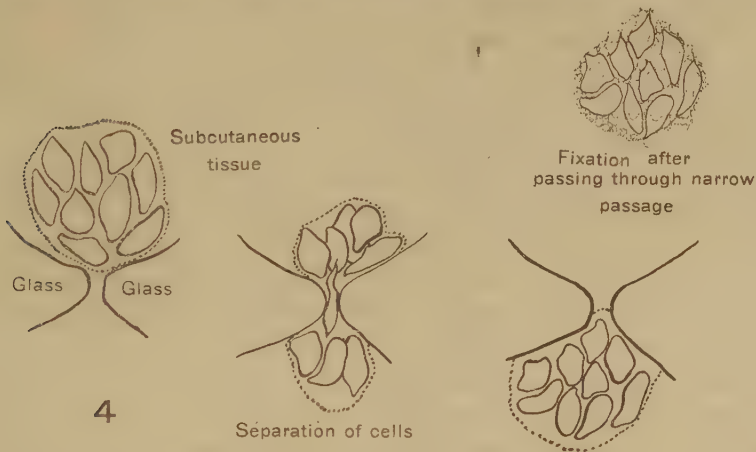


Fig. 4 Tissue from a four-day chick allowed to slip between two pieces of glass, while mounted under a cover-glass. After the entire mass squeezed through, one cell at a time, fixation shows the fibrils intact between all the cells. Camera-lucida drawings.

compressed, cell masses pushed out of their places, adhesions formed, all without leaving evidence of their original condition. This may be one way of interpreting the isolated endothelial-lined spaces and lymphatic anlagen described by Huntington ('10), McClure ('10), and Kampmeier ('12). The last points out (p. 430) that "histologically all incipient lymphatic anlagen . . . are decidedly different from either an active vein or a mature lymphatic. They lack definition and possess vague and undifferentiated outlines; for the cells of their walls are not arranged in that end-to-end fashion so characteristic of vascular

endothelia. Instead, many instances were observed under strong magnification where the tissue cells in their longest diameter stand perpendicular to the periphery of the anlagen and project far out into the lumen with their cytoplasmic filaments." Kampmeier interprets this condition as being brought about by the addition or fusion of contiguous spaces. However, these regions may have been continuous, and the apparent interruptions may have resulted from adhesions at the time of fixation.

The adhesive process may also describe the segmentation of the 'retrogressive venous channels' of different authors, in which the lumen of a vessel is interrupted by solid cell masses. The value of Kampmeier's observation (p. 433) that more delicate fibrils lie in the pathway of future lymphatics is evident, as it is probable that these represent regions of less concentration than the surrounding tissue, less precipitate having been left. Kampmeier (p. 451) further observes that "the elongation of lymphatic spaces and their fusion finally into a continuous channel, as well as the growth of their cavities in diameter is accomplished by the same process which gave origin to them, namely, by the disintegration of tissue fibrils and the concentric addition of spaces." The coagulated fibrils, however, as sections show, wall off spaces in one dimension only, while in the living condition the intercellular colloid is continuous throughout the region. The correspondence of the ages of the individual embryos in which these conditions are found indicates that the intercellular substance in certain definite places is in the same physiological condition.

Inasmuch as we can conclude from Kampmeier's observations that less dense regions form in the tissue and inasmuch as we have considered the mechanism by which adhesions can be brought about, we have a physiological basis for the distribution of growing lymphatics and blood-vessels. As the free-flowing blood and lymph are confined to vessels, walled in by endothelium, the growing ends of the proliferating capillaries probably follow the lines of least resistance and therefore take the less dense pathway through the tissues. It is conceivable that regions where oxidation of acids or their neutralization becomes defi-

cient, the tissue would absorb more water and eventually almost liquefy, allowing a growing capillary free access, and thus automatically establishing a better circulation for that part. The regions of finer fibrils in the pathway of growing capillaries strongly suggest this view. In fixed tissue it is not possible to make observations on the small changes in hydrogen ion concentration necessary to influence the tissues. These changes

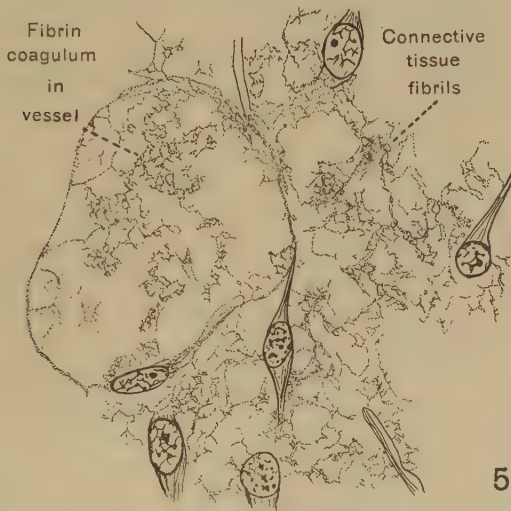


Fig. 5 Connective-tissue fibrils and fibrin in subcutaneous tissue of 55-mm. pig embryo. Fixed in Bouin. Stained in Mallory's connective-tissue stain and iron haematoxylin. Camera-lucida drawing.

may be exceedingly small, as shown by their influence on the secreting mechanism of excised kidneys (Isaacs, '17). Furthermore, 'young' capillaries of the blood and lymph system do not show concentric layers of connective tissue around them as do the larger vessels which have increased their size in situ, or more solid organs as the thyroid, thymus, or the salivary glands in the embryo, showing that little or no compression took place as the capillary grew in (figs. 1 and 5). This holds true, even though we take into account the contraction on fixation.

THE FIBER PRODUCING CELLS

Of cellular constituents, the spindle shape is apparently the more stable form. The multipolar forms can be considered as response forms caused by the conditions of the environment at any given moment. Ferguson ('12) and Clark ('12) have described the changes of shape of living connective-tissue cells, and their work points to the independent movement of these cells. Ferguson (p. 134) notes a change from round to stellate and stellate to round. In chick tissue, however, most of the cells take a short spindle form when surrounding tension and pressure is released. Ferguson's (p. 135) observation, that "the shape of the cell (stellate type) is undoubtedly influenced to some extent by its surroundings, and the duration of a particular stellate, spindle or lamellar shape may in some cases be thus determined," can be demonstrated by varying the pressure on the cover-glass in a tissue mount. His statement that "the general trend from round to stellate and from stellate to spindle is inevitable" is significant in indicating the changes of tension in the growing embryo. Rous and Jones ('16) describe a series of changes taking place in cells freed from connective tissue by digestion with 3 per cent trypsin solution. Under these conditions, the cells tend to become spherical. In our preparation we can also make the cells assume a more spherical form if any solution is added which contains more free water than the normal environment of the cells. This does take place of itself as soon as the water of syneresis forms in our preparations, as described before.

From the fact that in fresh mounts most of the multipolar or stellate cells on release from the tissue, before any stiffening takes place, assume the spindle forms, we can assume that the factors affecting the shape of these cells are the pulls and pushes affecting the region. Change in shape of a cell thus accompanies a change in surroundings. A comparison of the more compact mesenchymal tissue (greater number of nuclei per unit area) of a 10-mm. stage with that of a 30-mm. pig shows the latter to be looser, in spite of the fact that the growing internal organs

take up increased space and taking into consideration the contraction of the outer layers on fixing. Evidently the tension changes as the embryo grows. Clark ('12, p. 366) does not conclude that the change in position of individual cells can be accounted for only on a basis of general growth. When a piece of tissue is pushed with a needle under the microscope, the mucoid nature of the mass causes it to react as a whole, each cell being affected by the surrounding push just as much as the surrounding colloid. However, the cells are in a temporary stage of unstable equilibrium, and gradually work their way in the colloid until they have reached the most stable position for the new set of conditions. A demonstration of this process is seen in the descent of a piece of lead through a gelatin gel, or the conditions may be better illustrated with a watch spring embedded in gelatin of such a strength that the two bend together. After bending, the spring will eventually straighten itself, by working through the gelatin. This process, which is really diapedesis, is probably the mechanism by which tissues are shaped in response to pressure or tension stimuli.

As the tissue grows older, it becomes denser, the jelly becoming thicker, and response to pulls and pushes by permanent change in form less marked, because the cells have less freedom in the thicker jelly. Kaneko ('04) describes this in granulation tissue, in which the direction of the fibers which may be formed is influenced by the direction of stresses or pulls, while this response is lost in fully formed connective tissue.

It is of course a matter of general experience that embryos shrink in fixing or during the dehydration process. While this accounts for some of the compression of layers immediately underlying the skin and around the more solid organs, some is no doubt due to the fact that organs, as the glands, in their growth, glide into the connective-tissue jelly, which is first compressed and then readjusted to the new conditions. Sections often show the connective tissue compressed, yet separated by spaces from such organs as the salivary glands or thyroid, the space being bridged here and there by fibrils. This can be interpreted as indicating that there is no firmer union between

the connective tissue and the gland other than that of the general stickiness, due to the viscous intercellular substance. The relation of a gland to the surrounding connective tissue may be illustrated with gelatin solutions. A strip of a 4 per cent gelatin gel is immersed in a 2 per cent gelatin sol, and the latter allowed to gel, or it may be treated with a fixative. It will be found that the first strip, which is optically well marked off from its surroundings, retains its identity, and on being pulled out, retains some of the weaker gelatin sticking to it. However, this can be wiped off and the two separated. This expresses the relation of a growing gland to the connective tissue.

The ease with which embryonic connective-tissue cells (four-day chick) can be separated in the fresh condition indicates that their syncytial appearance is due to adhesion. Ferguson ('12) has observed the union of cell processes in living fundulus embryos, and Clark ('12) has mapped out their successive space relations in growing tadpoles. Under such circumstances, fibrils, if present, would either anchor the cells or else leave a visible trail of the cell passage. However, in sections they surround the cells on all sides, with no appearance as the tail of a comet, that we would expect under the circumstances. On killing the tissue, contraction and great shrinkage often results in the separation of the connective-tissue cells, so that many investigators, not being able to trace the connection from one cell to another, have concluded that the cells fade out into the fibrils.

THE 'FIBRILS' AND FIBER FORMATION

That the fibrils are artificial coagulation products may be inferred from the difference in delicacy of the pattern with different fixatives (fig. 2, B, D, E, F). Triepel ('11) notes this variation with the fixatives in studying connective-tissue fibrils and that of the coagulum in the blood-vessels in a given region in an embryo is somewhat the same. Triepel ('11) calls attention to the remarkable constancy of the pattern and its characteristic formation. This is of course natural, if the fibrillae are products precipitated by the fixatives. However, he attributes the different sizes of the fibrillar details to different amounts

of shrinkage caused by different fixatives in preexistent fibrils. Hansen ('99) recognizes 'pseudofibrillae' of cartilage as artifacts ('alcohol fibers' of Solger), but does not apply the principle to connective-tissue fibrils.

The fibrils take a golden-yellow color with orange-G, a light pink with acid fuchsin, and a blue with the aniline blue in Mallory's connective-tissue stain. With the latter, the bodies of the connective-tissue cells stain pink or orange-pink. Ferguson ('11) describes the collagenous fibrils as taking a golden-brown color with Bielschowsky's silver method. From the foregoing description of the origin of the fibrils as precipitation products, we can account for the variation in results with silver-impregnation methods. The fibrils cannot be demonstrated in the fresh mount with any of the above stains nor with the so-called vital stains, until subsequent changes, possibly dehydration, lead to fibril formation.

The more solid elements of the tissue including the white fibrous, the yellow elastic, and the precartilaginous tissue are formed from the intercellular jelly by the deposition of more material, making the jelly more concentrated, thus leaving more precipitate in fixed sections. The gelation and stiffening of the white and elastic fibers can be easily followed in the fresh subcutaneous tissue and tendons of the chick. Fresh preparations are mounted between a slide and cover, sealed, and examined immediately. Twelve-day chicks and those just hatched, illustrate the stages. While one watches through the microscope, slight pressure on the cover will serve to separate pieces of tissue. Elongated strands of tissue, stretching from piece to piece, may be seen with occasional spindle-shaped swellings. Analysis shows that these swellings represent connective-tissue cells, adhering to a stiff, jelly-like strand of the intercellular substance. On further separation, the strand apparently elongates. The fiber is very sticky at this stage. The older stages show that the connective-tissue cells are adhering closely to a well-formed fiber, and can be dragged along the fibers when tension is put upon them. The fiber is formed of the jelly between the cells, and the increase in toughness from a viscid state to a well-formed fiber is shown by

its changes of extensibility and consistency in the fresh and the varying intensity of the staining reactions in the fixed tissue. In the early stages, a well-formed young fiber can assume the appearance of a thick network, if it is treated with a coagulating or dehydrating fluid (fig. 6). In the later stages the jelly becomes thick enough, so that the holes remain when cells or muscle fibers are pulled out in manipulating the tissue. The sections indicate

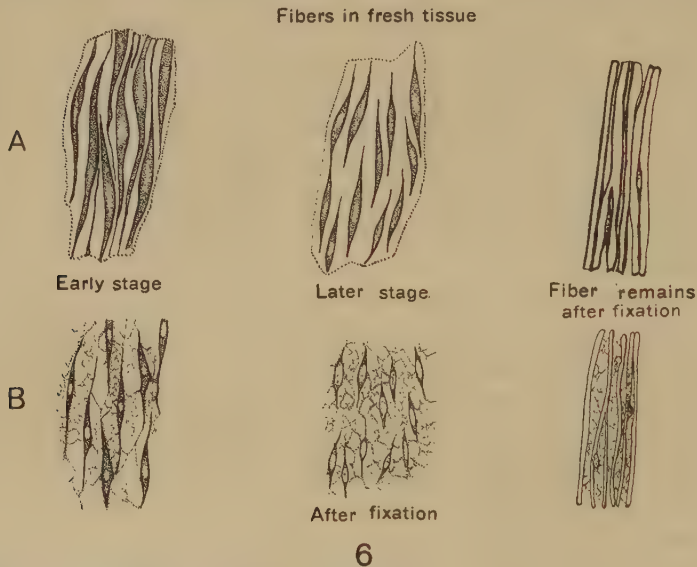


Fig. 6 Appearance of fibers in a sixteen-day chick, when flattened under a cover-glass. Successive ages are shown (A) before and (B) after fixing. Camera-lucida drawing.

a progressive increase in concentration due to deposition of more material, a fact shown by the increase in the amount of inter-cellular precipitate in sections. The fibers are first laid down close together in sheets or ribbons and separate into the familiar strands only after the expansion of the surrounding areas. Fixation, of course, separates them by shrinkage.

The speed of decolorization after staining is a factor in considering relative densities. The small fibrils lose their stain sooner than the larger and the white fibers last of all. A. Fisher ('99)

and Mann ('02), however, suggest that the greater relative surface of small particles over larger ones, in comparison to their volume, allows greater space for washing out of the stain. No fibrils can be demonstrated in the early or later stages, nor does Janis green, methylene blue, or neutral red show their presence. The edema set up by the use of aqueous solutions of these salts may be temporarily avoided by dusting a few grains of the powdered stain on the tissue. The diffusion of the stain brings about the result desired from the aqueous solution, by emphasizing the difference of refraction of the different constituents. As different authors have pointed out, the vital stains of this type act only on tissue which has already begun to die. Granules may dissolve some of the stain without killing the cell, however. *Paramoecium*, in which the posterior end is dead and consequently stained deep pink with neutral red, still retain their power of movement. *Fundulus* eggs can be grown in toxic solutions of substances, often, however, with the production of abnormalities. The continuation of one or more of the vital processes of a cell cannot be considered as a test of the normality, so that results with vital stains belong to the observations on experimental tissue, not necessarily normal.

The cells are probably the active agents in influencing the deposition of the material. The modern simile of an assembling and distributing plant probably describes the function of the cells in handling the materials in fiber formation. The movement and migration of the cells probably affect the distribution of the fibers and result in forming strands of the fibers, instead of one mass. The subsequent pulls and movements of the part as a whole cause the strands to glide over one another, and this is probably a second factor in the isolation of fibers. The appearance of fibers can thus be simulated in a gelatin or fibrin gel. There is some evidence to lead one to think that the cells are definitely polarized with respect to fiber-producing regions, thus accounting for the fact that some regions remain more jelly-like while neighboring regions around the same cell stiffen. The cell in profile is flattened on the fiber side, but convex on the jelly side.

Optical effects may be obtained with different concentrations of gelatin, giving the same contrast relations that are found in fresh tissues. If cubes of water-soaked gelatin of the same size are treated with dehydrating or hydrating agents of different strength (grades of alcohol, commercial formalin, Van Gehuchten's alcohol-acetic-chloroform, or corrosive sublimate), blocks of varying density are obtained. The greater the density, in this case, the greater the refractiveness (Isaacs, '16). In other words, the greater the density of a colloid of this type in the tissue, the greater its refractiveness and the lighter it appears when in focus under the microscope. It is for this reason that the cell nuclei and fibrils as well as other elements appear clearer when the preparation is allowed to stand and undergo coagulation and dehydration changes. The change is a real one, and is not merely due, as has often been suggested, to the eye becoming accustomed to the preparation.

The suggestion naturally follows that the fibrils may have been present as slightly more concentrated areas in the interstitial connective substance and escape detection while observed in the fresh tissue. Maxinow ('06) describes the ground substance as homogeneous, with granules which probably represent a network. Danchakoff ('08) considers that the spaces left in sections are due to extraction or dissolving out of the intercellular substance. While considering the action of reagents as accounting for some granular deposits, Danchakoff describes the fibrils as cell processes. However, the precipitating action of reagents can be seen under the microscope by applying them with a delicate pipette to the under surface of a hanging-drop preparation, thus avoiding the danger of 'washing out.' The action is seen to be one of condensation and precipitation of the dissolved material, leaving the fluid part in the meshes of the resulting granular coagulum. The results can be checked up with stains.

Höber ('14) states that structures produced in gelatin by alcohol are not preformed, but are produced on dehydration. In order to see if the fibrillae were preformed or were artifacts, the tissue (skin, subcutaneous tissue, or muscle of a chick embryo) was pressed free from blood and lymph, and then irrigated with

a potassium oxalate salt solution (Ringer's solution with potassium oxalate substituted for the calcium chloride, an empirical solution) and the solution filtered. A similar solution can be made by allowing connective tissue to stand overnight in a little Ringer's solution. Treating a drop of this solution, after filtering, with absolute alcohol or Zenker's solution on a slide, a complete network, resembling that of the tissue fibrils, was obtained, and it took the fibrillar stains (fig. 2, A). Extracts from most tissues can be precipitated in the same way, giving fibrillar structures characteristic of each tissue. The fact that a complete network was obtained in this case would seem to indicate that the fibrillar network was an artificial precipitation product. Fixation of the washed tissue shows a decrease in the number of fibrils. The substance which was filtered evidently contained material from the more fluid intercellular substance. It is to be expected that this contained the same serum albumin, serum globulin, and fibrinogen that we normally find in the blood and lymph, and this in the end is probably the key to the network formation between the connective-tissue cells. The presence of some mucin-like substance alters the staining reaction somewhat and enables us to differentiate it from pure lymph coagulum. A similar substance and a similar network may be encountered in any tissue. The network bears the same relation to the intercellular jelly as the crystal colony bears to the solution from which it develops and is specific for each of the different colloids under the same conditions.

Fleming ('97) and others maintained that the fibers were transformations of the cell protoplasm, Meves ('10) specifying their origin from chondrioconta at the cell surface.

FRAMEWORK OF ORGANS

The digestion method of demonstrating fibrils, as applied by Mall ('92) and others, takes advantage of the fact that the fibrils apparently resist pancreatic digestion in alkaline solution. Mall ('02) finds that unfixed, frozen sections which are digested are difficult to stain in any satisfactory way, due to mechanical difficulties. He obtained a better picture in alcohol-fixed tissue.

Flint ('04) suggests the use of alcohol-chloroform-acetic acid, sublimate acetic, or alcohol alone to show the 'fibrillar framework' of organs by pancreatic digestion. Formalin cannot be used for this purpose. It will be noticed that those reagents best suited for this demonstration coagulate the homogeneous connective-tissue colloids under the microscope into the hard definite connective-tissue fibrils. Zenker's solution, while showing the fibrils, presents secondary difficulties which bar its use in digestion work. Sublimate solutions and chromium salts cannot be used advantageously in studying connective tissue, as the coagulated colloids fringe the cells with fibrils, thereby covering up many details.

Fresh tissues exposed to several changes of an alkaline solution of pancreatin for varying lengths of time (from days to weeks) without any preservative, but conducted under aseptic conditions, do not show the fibrillae when mounted under the microscope. Instead, we have a uniform jelly between the white fibers and the spaces occupied by the cells. The fibrillae, however, can be made to appear by dehydrating or coagulating agents. This enables us to interpret Mall's ('02) results when he finds that the digestion method "causes the sections, if fresh, to become a swollen and slimy mass in which the delicate fibrils can be seen after it is treated with picric acid." Picric acid precipitates the fibrils from solution. A consideration of the following test-tube experiments may be helpful in this connection. If fresh albumin is digested in an alkaline solution of pancreatin, a clear solution results. The addition of alcohol or sublimate acetic results in a flocculent precipitate (peptones). Therefore, if any product of digestion remain in the homogeneous jelly resulting from digestion, we can have just as complete a network formed as if no digestion took place. Posner and Gies ('04) point out that the "connective-tissue mucoids are readily digested by trypsin in alkaline solution." If the washing is complete enough to remove the products of digestion, then the tissue falls to pieces and the results are considered 'unsatisfactory.' The unreliability of digestion methods is a part of the experience of all who have used them. This would indicate the possibility that the fibrillar details in the

framework of organs and basement membranes may be products of fixation. Mall ('92), Flint ('04), and Moody ('10), among others, give excellent descriptions of such digestion preparations, which, if considered from the point of view of coagulation products, indicate something of the distribution of the intercellular colloid.

NEUROGLIA AND THE INTERCELLULAR JELLY OF THE NERVOUS SYSTEM

The jelly-like nature of fresh nervous tissue, as the cerebral hemispheres of the adult frog or its medulla, is a constant characteristic. This tissue when mounted fresh between a slide and cover and sealed shows a field of cells, nuclei, and nerve fibers imbedded in a clear homogeneous jelly.¹ By varying the pressure, different details can be brought out. If some alcohol is allowed to run under the cover, the picture changes entirely. A heavy groundwork of very delicate fibrils develop both in the tissue and in the expressed jelly surrounding it. The nerve fibers often act as bases around which and from which the fibrils radiate. Van Gehueten's fluid gives an equally heavy crop of fibrils. The presence of different structures, as capillaries, active ciliated cells, and nerve fibers, serve often to give a clue as to just what part of the brain wall we are studying.

Hardesty ('04) points out that the development of the neuroglia fibers is a process of transformation of fibrillated areas. The deeply stained fibers in the exoplasm of the syncytium of his sections are seemingly derived from a condensation of the less deeply staining substance. However, a study of the fresh tissue leads to the conclusion that this described formation is really the result of precipitating the successive stages with the fixative. The increase in concentration and density brought about by addition and deposition of more material to the jelly gives us a basis for variations in the pictures obtained in successive stages. Coagulation on fixation, then, would leave a more compact mass where the fibers are, but a delicate network ("fine threads of the spongioplasmic network" (p. 262)) in the less concentrated parts. This work corroborated Weigert's and Hardesty's (p. 257) conclusion that "the fibers cannot be regarded in any sense as out-

growths of the cells," but on the other hand it indicates that we are dealing with more or less concentrated colloids of the homogeneous intercellular substance and that the fibrillated appearance of the so-called exoplasm is a fixation product. Holmgren ('04) and later Ross ('15) have described prolongations of cytoplasmic processes of glia cells, which appear in section to run into the 'trophospongia' of the nerve cells. These apparent "non-nervous partitions of capsular processes continuous with the glia cell" are in reality the remains of the intercellular jelly which when coagulated by the fixative or in postmortem processes appear to be fine protoplasmic fibrillae continuous with the glia cells on the one hand and the trophospongia on the other.

SUMMARY AND CONCLUSIONS

The intercellular jelly of embryonic and adult tissue is structurally homogeneous and contains no network of fibrils. The evidence may be summed up as follows:

1. Fibrils cannot be seen in the living intercellular substance.
2. Fixatives, drying, dehydration, or coagulating reagents are necessary to show the fibrillae.
3. In young embryos the cells may be rearranged by manipulation of the tissue, but on fixation the fibrils are continuous.
4. The process of fibril formation can be followed under the microscope.
5. The possibility of 'washing out' a non-coagulated colloid from the meshes of a network can be eliminated by fixing the tissues under the microscope.
6. The form and structure of the network varies with the fixative.
7. Cut pieces of tissue placed in contact and fixed show a continuity of fibrils.
8. Intercellular jelly washed out and passed through a filter can be precipitated as a complete network with the ordinary fixatives.
9. Complete washing out of the intercellular jelly gives a fibrillar-free picture when the tissue is treated with fixatives, while the filtrate can be made to precipitate as a fibrillar network.

10. Digestion methods do not show the fibrils unless some step in the technique involves a coagulating or dehydrating process.

11. Complete and similar fibrillar networks can be obtained by the action of fixatives on pure solutions of gelatin, mucin, plasma, egg-albumin, and other solutions.

12. While the density of the network increases with the age of the tissue, the process is reversed when postmortem digestion or acidosis is allowed to proceed. The state of the colloid at the time of fixation determines the type of fibrils.

13. Cells may move freely in certain embryonic stages, and sections show no track left by the passing cell in among the fibrils.

14. In fixed and sectioned tissue the cells and their processes and fibers show by their alignment the evidence of pressure or pulls. The 'fibrils,' however, radiate in all directions unchanged and do not show stress lines.

The consideration of connective tissue and neuroglia fibrillae as fixation artifacts is of aid in accounting for the following phenomena:

1. Movement of cells, Diapedesis. (The pathway is a structureless jelly.)

2. Progressive increase in strength with age, from the jelly-like younger embryos to the tougher adult tissues.

3. Non-appearance of fibrillae in the living, with their appearance in fixed tissue.

4. The variation in the fibril pattern when different fixatives are used.

5. The similarity of pattern of fibrin in the blood-vessels and fibrillae between the cells.

6. The similarity of many of the staining reactions of the fibrillae and fibrin. Those stains which stain the mucoid element serve to differentiate.

7. Accommodation of the connective tissue to the invading cells of growing organs.

8. The appearance of isolated, fluid-filled spaces lined by endothelium in the connective tissue.

9. Variation in the behavior of successive sections or 'similarly' treated pieces of tissue when subjected to pancreatic digestion.

10. 'Superiority' of fixed tissue over fresh tissue for demonstrating "fibrillar structures of frameworks of organs" by means of digestion methods.

11. The variation of behavior of fibrils to Bielschowsky's silver method.

12. The appearance of fixed tissue of cells, much smaller than when alive, apparently fading out into fibrillae.

13. The clear-cut lines of separation when connective tissue shrinks away from the more solid cell masses on fixation, leaving a few fibrillae bridging the gap.

14. The stickiness of living connective-tissue substance and connective-tissue cells.

15. The increase in density of the fibril network with age. The more concentrated a colloid, the thicker the network that is formed on precipitation.

16. The varying observations on basement membranes.

17. The appearance of ribbon-like fibers in the fresh, which turn into a thick network of fibrils on fixation.

18. The appearance of neuroglial fibrillae ('cell processes') extending into trophospongia of nerve cells. The precipitation of the intercellular colloid is a simpler explanation.

The fibers of adult tissues are formed by the thickening (concentration increase) of the colloid lying between the fibroblasts. The polarization of the cells, their movement, and the stress exerted on the growing tissue, all serve to give the adult white fibers their arrangement as strands in a bundle. This method of fiber formation enables us to understand the shrinkage which accompanies fibrosis in the tissues. If we accept the fact that a less dense colloid leaves lighter fibrils than a more concentrated one, then we have a means of telling the consistency of tissues when the fixed sections are studied. A physiological determinant is also supplied, directing the distribution of new capillaries along the lines of least resistance.

LITERATURE CITED

- BAITSELL 1915 The origin and structure of a fibrous tissue which appears in living cultures of adult frog tissues. *Jour. Exp. Med.*, vol. 21, p. 479.
- BELL 1909 On the histogenesis of adipose tissue of the ox. *Am. Jour. Anat.*, vol. 9, p. 420.
- BÜTSCHLI 1892 *Mikroskopische Schäume*. Leipzig.
- CLARK, E. R. 1912 Further observations on living growing lymphatics: their relation to the mesenchyme cells. *Am. Jour. Anat.*, vol. 13, p. 360.
- DANCHAKOFF 1908 Untersuchungen über die Entwicklung von Blut und Bindegewebe bei Vögeln. *Arch. f. mikroskopische Anatomie u. Entwicklungsmechanik*, Bd. 73, S. 147.
- FERGUSON 1911 The application of the silver-impregnation method of Bielschowsky to reticular and other connective tissues. *Am. Jour. Anat.*, vol. 12, p. 277.
- 1912 The behavior and relation of living connective-tissue cells in the fins of fish embryos, with special reference to the histogenesis of the collagenous or white fibers. *Am. Jour. Anat.*, vol. 13, p. 129.
- FISCHER, A. 1899 *Fixirung, Färbung und Bau des Protoplasma*. Jena, S. 34-36.
- FISCHER, M. H. 1915 *Oedema and Nephritis*, 2nd ed., New York, p. 240.
- FLEMMING 1897 Quoted from Schäfer: *Textbook of Microscopic Anatomy*, 11th ed., New York, 1912.
- FLINT 1904 The connective tissue of the salivary glands and pancreas with its development in the glandula submaxillaris. *Johns Hopkins Hospital Reports*, vol. 12, p. 8.
- HANSEN 1899 Über die Genese einiger Bindegewebsgrundsubstanzen. *Anatomische Anzeiger*, Bd. 16, S. 424.
- HARDESTY 1904 On the development and nature of neuroglia. *Am. Jour. Anat.*, vol. 3, p. 254.
- HARDY 1899 On the structure of cell protoplasm. *Jour. of Physiol.*, vol. 14, p. 187.
- HÖBER 1914 *Physikalische Chemie der Zelle und der Gewebe*. Leipzig and Berlin, S. 313.
- HOLMGREN 1904 Über die Tropospongien der Nervenzellen. *Anatomischer Anzeiger*, Bd. 24, S. 225.
- HUNTINGTON 1910 The phylogenetic relations of the lymphatic and blood-vascular systems in vertebrates and the genetic principles of the development of the systematic lymphatic vessels in the mammalian embryo. *Anat. Rec.*, vol. 4, p. 1.
- ISAACS 1916 Properties of colloids in relation to tissue structure. *Anat. Rec.*, vol. 10, p. 517.
- 1917 The reaction of the kidney colloids and its bearing on renal function. *Am. Jour. Physiol.*, vol. 45, p. 71.
- KAMPMEIER 1912 The development of the thoracic duct in the pig. *Am. Jour. Anat.*, vol. 13, p. 430.
- KANEKO 1904 Künstliche Erzeugung von Margines falciformes und Arcus tendinei. *Arch. f. Entwicklungsm.*, Bd. 18, S. 317.

- MALL 1892 Reticulated tissue and its relation to the connective tissue fibrils. Johns Hopkins Hospital Reports, vol. 1.
1902 On the development to the connective tissues from the connective tissue syncytium. Am. Jour. Anat., vol. 1, p. 331.
- MANN 1902 Physiological histology, Oxford, p. 106.
- MAXINOW 1906 Über die Zellformen des Lockeren Bindgewebes. Arch. f. mikroskopisches Anatomie, Bd. 67, S. 683.
- McCLURE 1910 The extra-intimal theory and the development of the mesenteric lymphatics in the domestic cat. Anatomischer Anzeiger, Bd. 37, S. 101.
- MEVES 1910 Über Strukturen in den Zellen des embryonalen Stützgewebes sowie über die Entstehung der Bindegewebsfibrillen, insbesondere derjenigen der Sehne. Arch. f. mikroskopischen Anatomie, Bd. 71, S. 149.
- MOODY 1910 Some features of the histogenesis of the thyroid gland in the pig. Anat. Rec., vol. 4, p. 429.
- POSNER AND GIES 1904 A preliminary study of the digestibility of connective tissue mucoids in pepsin-hydrochloric acid. Am. Jour. Physiol., vol. 11, p. 350.
- RANVIER 1889 Quoted from Schäfer: Textbook of microscopic anatomy, 11th ed., London, 1912, p. 117.
- ROSS 1915 The trophospongium of the nerve cell of the crayfish (Cambarus). Jour. Comp. Neur., vol. 25, p. 523.
- ROUS AND JONES 1916 A method for obtaining suspensions of living cells from the fixed tissues and for the plating-out of individual cells. Jour. Exp. Med., vol. 23, p. 549.
- SCHÄFER 1912 Textbook of microscopic anatomy, 11th ed., London, 1912, p. 116.
- TRIEPEL 1911 Das Bindegewebe im Schwanz von Anurenlarven. Arch. f. Entwicklungmekhanik, Bd. 32, S. 482.